JOURNAL OF DAIRY SCIENCE

APRIL, 1956

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ANNUAL MEETING

June 19-21, '56

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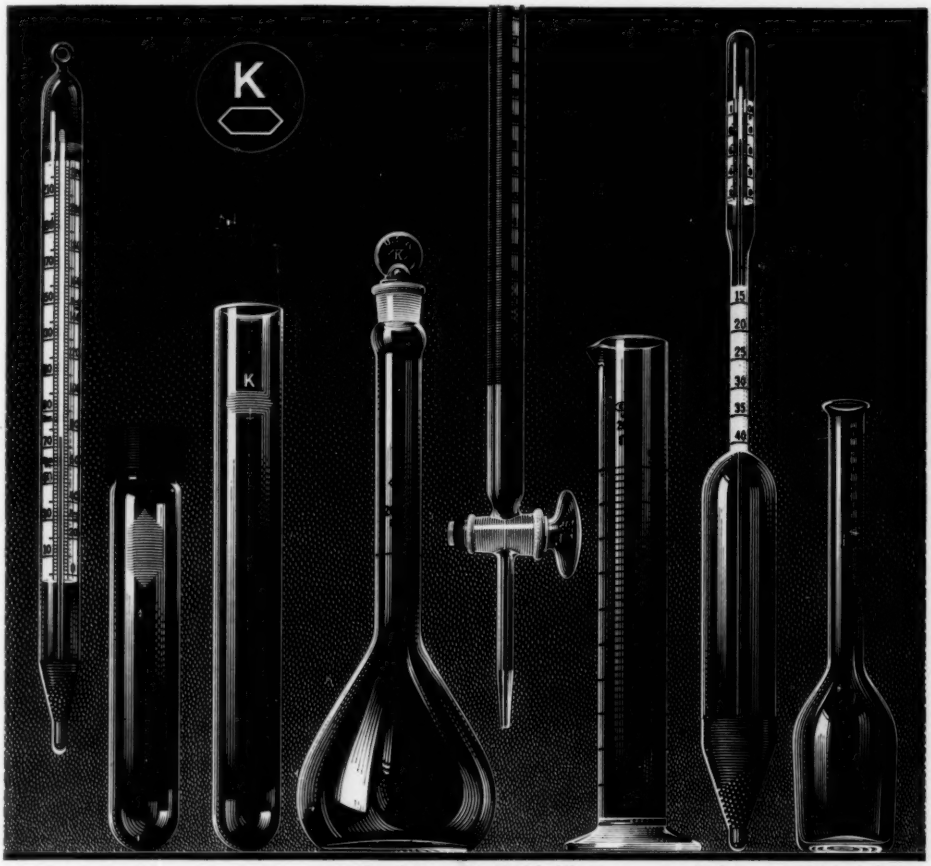

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Changes of Address

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Subscriptions for the British Isles and the British Empire should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Street, Covent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert Street, St. Leonards, N.S.W., Australia.

Membership dues in the Association are $10.00 a year and should be sent to the secretary-treasurer at the address above. Renewals should be made not later than December 1 each year.

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PEOPLE and EVENTS
in the Dairy Science World

Activities of the Divisions of A.D.S.A.

As a part of the program of the American Dairy Science Assoc. during its 50th anniversary year, special attention is being given to the activities of the three divisions of the Association: the Eastern, Southern, and Western. A special committee composed of H. L. WILDASIN, Massachusetts; W. A. KING, South Carolina; and J. O. YOUNG, Oregon, has been organized for the purpose of recommending ways and means of integrating to a fuller degree the work of the three divisions and the parent group.

The divisions were organized many years ago for the purpose of developing effective programs in education and science of special interest to members in the particular region. They operate within the framework of the constitution of the parent association. Reports of their activities are made for publication in the *Journal of Dairy Science*. All divisions are active in sponsoring student cattle and products judging contests, held at prominent expositions within their respective areas.

One or more officers of the parent association attend division meetings. Secretary-treasurer H. F. JUDKINS attended a recent meeting of the Southern Division in Atlanta, held in conjunction with the meeting of Southern Agricultural Workers. Papers on breeding, feeding, herd management, and milk technology were presented. A feature of the meeting was the banquet held with the Atlanta Dairy Technology Society, at which former GOVERNOR TALMADGE was the principal speaker. C. E. WYLIE, head of the Dairy Dept. at the Univ. of Tennessee, was honored by his colleagues as the Southern Division's Dairyman of the Year.

The officers of the Southern Division are: president, H. C. OLSON, Oklahoma A & M; vice-president, K. M. AUTREY, Alabama Polytechnic Institute; and secretary-treasurer, J. W. POU, No. Carolina State College.

The Western Division holds its meeting in connection with the Western Section of the American Society of Animal Production. At their July 1955 meeting at Laramie, Wyoming, attendance was 148 from 18 states, the District of Columbia, and Hawaii. The 1956 meeting is to be at the Univ. of Nevada, Reno, July 15-18. The officers of the Western Division are: president, J. O. YOUNG, Oregon State College; vice-president, L. R. HUNSAKER, Utah State College; and secretary-treasurer, I. W. SLATER, Univ. of Wyoming.

The Eastern Division holds its meeting in connection with the Eastern States Exposition, Springfield, Massachusetts, in September. The officers of the Eastern Division are: president, G. W. TRIMBERGER, Cornell Univ.; vice-president, H. L. WILDASIN, Whiting Milk Co., Charlestown, Mass.; and secretary-treasurer, R. E. WARD, Eastern States Farmers Exchange, West Springfield, Mass.

Intern. Dairy Congress to Be Held in September

The 14th Intern. Dairy Congress is to be held at Rome, Italy, Sept. 24-28, 1956, according to DR. R. E. HODGSON, Chief of the Dairy Husbandry Research Branch of the Agricultural Research Service, who is chairman of the U. S. Committee for the Congress. Those planning to attend should send in their registrations by May 31 to "Segretariato General del XIV° Congresso Internazionale Sul Latte E Derivati, via Guidubaldo Del Monte, 24, Roma." The registration fee is 12,500 lire.

Proceedings of the Congress will be published in English, French, German, Italian, and Spanish.

Florida Announces Industry Meetings

The Dept. of Dairy Science of the Univ. of Florida in cooperation with the Florida Dairy Assoc. has announced the following industry meetings:

July 19-20, Dairy Field Day. For milk producer-distributors, milk producers, dairy processors, herdsmen, county agents, vocational agriculture teachers, veterinarians, DHIA workers, and equipment and supply dealers.

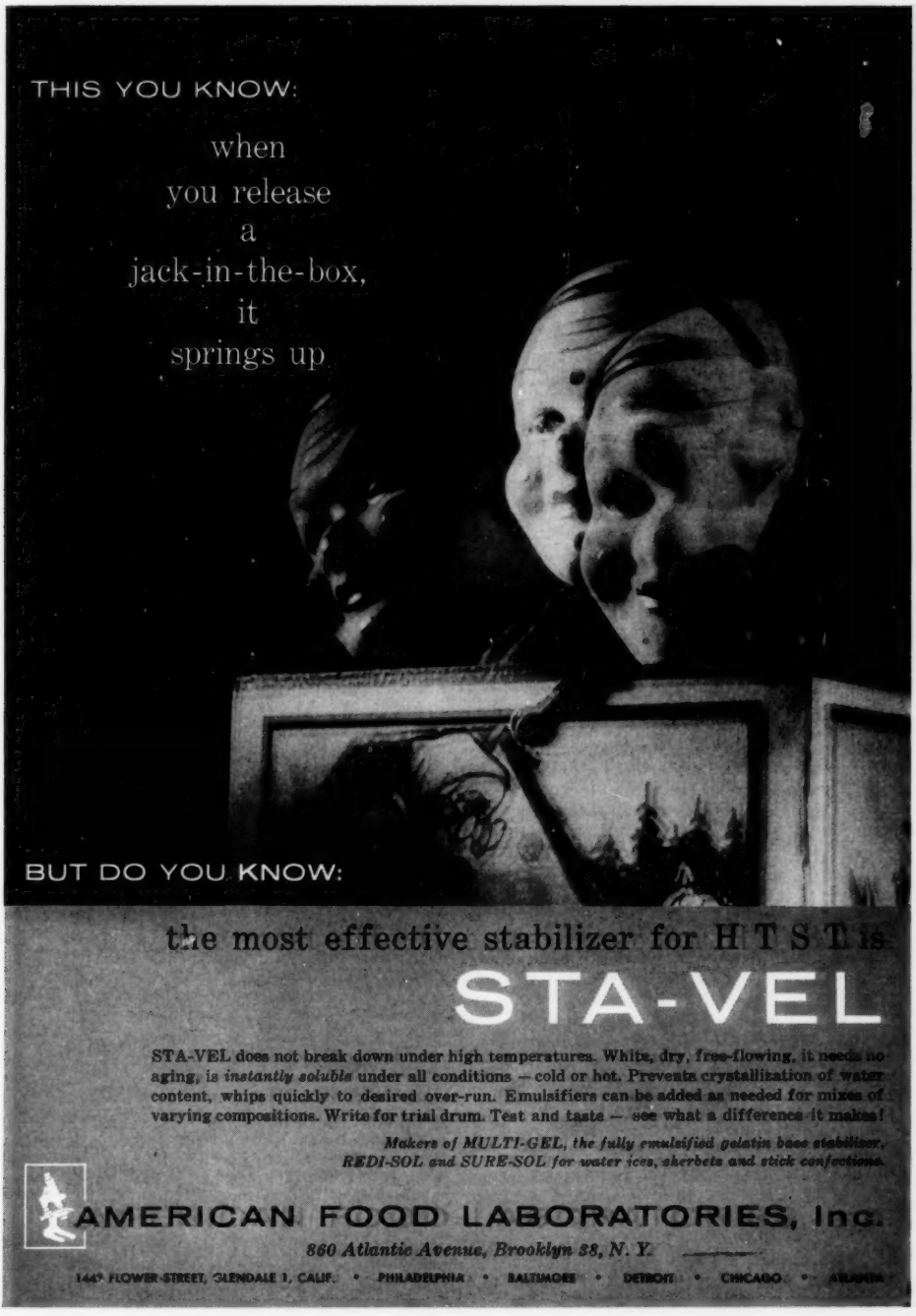
Aug. 21-23, Dairy Herdsmen's Short Course. For dairy herdsmen, herd owners, dairy farm helpers, DHIA supervisors, producer-distributors, and milk producers.

Oct. 11-13, Dairy Plant Operators' Short Course. For dairy plant superintendents and assistants, managers, owners, dairy plant employees, producer-distributors, and equipment and supply dealers.

New Constitution Approved

The vote on approval of the Association constitution and by-laws as they appeared in the October, 1955, issue of the Journal was as follows:

Number affirmative, 445
Number negative, 6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North Carolina Happenings

A conference for milk sanitarians was held at N. C. State College Feb. 23. The theme of the meeting was "The Effect and Use of Milk Sanitarians' Time." Speakers were C. A. ABELE, Diversey Corp.; R. P. MYERS, U. S. Public Health Service; HOWARD GOFORTH, Coble Dairy Products Coop.; and J. M. JARRETT, N. C. State Board of Health.

A short course for dairy laboratory technicians was held Feb. 20-23. The course was designed as a refresher for personnel employed in regulatory armed services and industry laboratories. Lecture and laboratory instruction was given on the standard plate count, direct microscopic count, coliform count, and phosphatase test. On the afternoon of the last day a round-table discussion was held jointly with milk sanitarians, the subject being "The effective use of the laboratory in quality milk control." Special assistance was given by R. P. MYERS, of the U. S. Public Health Service, Cincinnati, Ohio.

L. A. MAYNARD, head, School of Nutrition (retired), Cornell Univ., is on the campus of N. C. State College as a visiting professor. His seminars, personal advice, and informal discussions are enlightening and stimulating to all who come in contact with him.

The fifth annual Dairymen's Conference was held Feb. 21 and 22. Approximately 400 dairymen and guests heard discussions on culling, milk fever, milk flavors and odors, current research results, and silage preservatives. J. G. HAYS, extension dairyman, retired, Michigan State Univ., was the featured luncheon speaker.

J. J. MCNEILL joined the Dairy Husbandry staff as assistant professor in January. He spent 3 years as an undergraduate student at George Washington Univ., after which he attended the Univ. of Maryland, where he received his B.S. and M.S. degrees and will receive his Ph.D. degree in June, 1956. At N. C. State he will devote his efforts to studying the metabolism of rumen bacteria with emphasis on hydrogen and methane metabolism. He is working also on the effect of antibiotic feeding on ruminants and rumen bacteria.

Dairy Industry Members to Meet

The official slogan for the largest industrial show of 1956, the 20th Dairy Industries Exposition, will be "Frontiers for Profit!"—for profit frontiers are what some 25,000 dairy industrial men and women will find all around them during the week of Oct. 28–Nov. 3, in Convention Hall in Atlantic City, N. J.

Dairy Industries Society, Intern., will hold its tenth annual meeting at the Hotel Traymore, Oct. 28. Intern. Assoc. of Ice Cream Manufacturers will hold its 52nd annual convention, with the Shelburne and Dennis Hotels serving as headquarters, October 29-31. Milk Industry Foundation will hold its 49th annual convention at the Chalfonte–Haddon Hall Hotels, Oct. 31–Nov. 2. National Assoc. of Retail Ice Cream Manufacturers will hold its annual convention at the Shelburne and Dennis Hotels, Nov. 1-3.

Special events of particular appeal already planned include:

The 35th Collegiate Students' Intern. Contest in Judging Dairy Products, in which teams from the leading dairy colleges from the United States and Canada will compete for cash fellowships. Contest is cosponsored by A.D.S.A. and DISA.

Daily social hours in two special Exposition areas—the International Lounge, where visitors from abroad may receive free translation services and technical advice; and the Old Timers' Lounge, where veterans of 25 years or more of service to the dairy industries will be honored.

Special salutes daily to commemorate achievements—such as the passage of the Pure Food and Drugs Act in 1906—or to publicize valuable aspects of industry progress—such as the widening of the 3-A Sanitary Standards for Dairy Equipment program—or to direct attention to all-industry problems, such as that of adequate trained personnel for an increasingly technical industry.

Ohio News

At the meeting of the 23rd Annual Dairy Technology Conference held Feb. 9-10 at The Ohio State Univ. T. V. ARMSTRONG, assistant professor of dairy technology and a member of the staff since 1937, was honored for his service to the dairy industry. Alumni, friends of the industry, and his associates of the University joined to present "Army" with gifts and a scrapbook of letters and pictures.

T. V. Armstrong

Professor Armstrong graduated in 1921 from Ohio State. In the department he is in charge of the audit program and the Ohio examination of weighers, samplers, and testers; is responsible for the analytical service of the department, and conducts research on milk and milk products.

Approximately 500 persons attended the conference. Thirty-five visiting speakers discussed problems in the areas of farm milk production, buttermaking, market milk, engineering, ice cream, and concentrated milk. The theme of the conference was "Highways to Efficiency." Climaxing the conference was the banquet, co-

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operatively sponsored by the Department and the four Ohio dairy technology societies. DR. GENE STONE, general secretary of the Intern. Christian Endeavor, Columbus, spoke on "More Power to You."

W. ("BILL") WEBB, graduate from O. S. U. Dept. of Dairy Technol. in 1950 and executive secretary of the Columbus Milk Dealers Assoc. since 1952, assumed the position of assistant executive secretary in the Columbus Academy of Medicine. His successor in C. M. D. A. is LUTHER SIMMONS, graduate of O. S. U. in 1950 in the Dept. of Dairy Science. He has been a member of the Health Department staff in Cambridge, Ohio.

C. L. HANKINSON has been appointed associate professor in the Dept. of Dairy Technology, effective March 12. His duties will be to teach and conduct research on market milk and allied subjects. Dr. Hankinson received his undergraduate training at Michigan State Univ., earning his B.S. degree in 1934, his M.S. at the Univ. of Connecticut in 1935, and his Ph.D. at the Univ. of Minnesota in 1940.

Since 1940 Dr. Hankinson has been active in the industrial dairy field, having served at various times as research chemist and group leader for the Carnation Milk Co.; research director, manager, secretary, and director of North American Creameries; and as manager of the Kentucky Ice Cream Co. He is author and coauthor of several scientific articles covering the fields of curd tension of milk, hemolytic bacteria in milk, and the amylase enzyme system in milk. He holds two patents, one on the purification of rennin and the other on whipping compound from milk proteins.

Dr. Hankinson served as chairman of the manufacturing section of A.D.S.A. for 2 years, was the first president of the Minnesota Dairy Technology Society, and is a member of the Research Council of the American Poultry Institute.

L. S. HOLLINGER of Orrville is the 1956 president of the Ohio Dairy Products Assoc. Other officers are: vice-president, RICHARD MCCLELLEN, Lancaster; treasurer, C. T. ALEXANDER, Columbus; and executive secretary, FRED GREINER, Columbus.

DISA Elects Officers

S. E. CROFTS, Batavia Body Company, Batavia, Ill., was elected president of Dairy Industries Supply Assoc., as the trade group of approximately 420 dairy industrial supply and equipment firms brought their 37th annual meeting to a close at the Hotel Commodore in New York City on March 2. Previously the vice-president, Mr. Crofts succeeds L. N. LUCAS, the Bastian-Blessing Co., Chicago, who had served as president since 1954. D. G. COLONY, Manton-Gaulin Mfg. Co., Everett, Mass., was elected to the vice-presidency. R. E. CAIRNS, Waukesha Foundry Co., Waukesha, Wis., was reelected treasurer.

At the closing luncheon, the retiring president, L. N. Lucas, was presented with a large scrapbook containing clippings, photographs, and other mementos of his years of service to the Association. On the preceding evening, K. L. WALLACE, A.P.V. (Canada) Equipment Ltd., retiring from the Board after 18 years of service, including two as president and four as chairman of the Executive Committee, was awarded DISA's Honor Plaque for outstanding service to the Association. Only eight such plaques have been awarded.

Arrangements for the meeting were made by a committee headed by VINCENT RABUFFO, *Ice Cream Trade Journal*, and composed of J. C. DAVIS, American Seal-Kap Corp.; R. O. DAVISON, Kelco Co.; NORMAN MYRICK, Urner-Barry Co.; and V. K. SHUTTLEWORTH, American Can Co.

Univ. of Kentucky News

More than 160 sanitarians, fieldmen, producers, and dairy plant managers attended the Fieldmen's and Dairymen's Short Course at the Univ. of Kentucky, Feb. 20-22. The course was sponsored jointly by the Univ. Dairy Section, Kentucky Cream Improvement Assoc., Kentucky Manufactured Milk Improvement Assoc., Kentucky Purebred Cattle Assoc., and Kentucky Assoc. of Milk and Food Sanitarians.

Among the problems discussed were those of water supply on dairy farms, various methods of pasture and weed control, use of bulk tanks, and the use of silage in dairy cattle feeding. H. B. HENDERSON, chairman of the Dairy Division of the Univ. of Georgia, was the featured speaker at the annual banquet held in conjunction with the short course. He spoke on recruitment of personnel in the dairy field and the need for a commercial scholarship program. It was announced at this conference that the Kentucky Manufactured Milk Improvement Assoc. had voted to grant two $200 scholarships to men interested in dairy manufacturing.

At the annual meeting of the Kentucky Assoc. of Milk and Food Sanitarians the following officers were elected: L. E. SMITH, Kentucky State Dept. of Health, president; F. K. BALL, Milk Sanitarian, Newport, vice-president; and FRANK OSBORN, Falls City Milk Producers Assoc., secretary-treasurer.

A "Weighpail" for Pipeline Milkers

A new stainless steel weighing device called the "Weighpail" may well solve the problem of weighing and test-sampling each cow's production in modern milking parlors. By getting rid of easy-to-break and hard-to-clean glass weigh jars, the trend to parlor milking will be speeded along, herd testers believe.

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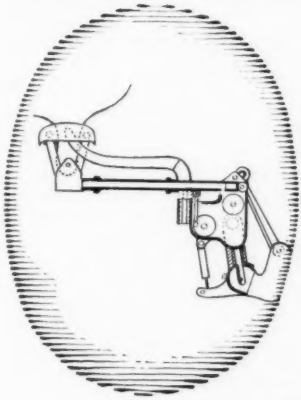

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News from West Virginia

G. A. CAIRNS, dean of the College of Agriculture, Univ. of Maryland, conducted a 2-day judging school at W. Va. Univ. April 20-21. The judging school, under the sponsorship of the Dairy Department, was requested by the state Dairymen's Assoc. for the purpose of training county workers, leading dairymen, and others to do a better job of officiating at county and community fairs throughout the state. The Dairy Cattle Congress gives W.V.U. students practical training in fitting and showing and in conducting a dairy show.

JACK NESBIT, Columbus, Ohio, will be the principal speaker at the annual W.V.U. Dairy Day activities to be held at the Dairy Farm, Morgantown, June 28. This event, which usually attracts more than 1,000 visitors, includes demonstrations on the latest dairy methods and a look at the dairy herds maintained at the University.

Dairy Association Honors C. S. Rhode

C. S. RHODE, professor emeritus of dairy science extension of the Univ. of Illinois, was honored at the annual meeting of the Illinois Purebred Dairy Cattle Assoc. Feb. 24. A native of Indiana and a Purdue graduate, Rhode has been a member of the University's dairy staff since 1916. He was honored for his outstanding contributions to dairying in Illinois.

He was one of the first extension dairymen in the country to set up an owner-sampler testing program. From the time extension work was started in Illinois, Rhode has stressed the importance of outstanding herd sires. In 1922 he organized the first cooperative bull association in Illinois, and in 1924 he started the Illinois Bull Exchange Plan.

In 1940 he assisted with the organization of a cooperative artificial breeding association to serve the northern half of Illinois. A similar organization for the southern half of the state was organized in 1945. He has acted in an advisory capacity and has assisted in the sire selection and the breeding programs that followed. More than 250,000 dairy cows were artificially bred last year.

Rhode is an official classifier for the Brown Swiss Breeders' Association and has judged most of the prominent national and international dairy cattle shows.

Judges Announced for International Show

Six well known judges will officiate at the 4th Intern. Dairy Show, which will be held in Chicago in the Intern. Amphitheatre Oct. 6 to 13. G. A. CAIRNS, Univ. of Maryland, will judge Ayrshires; Brown Swiss are assigned to HAROLD KAESER, Ohio State Univ.; the Guernsey classes will be judged by WILLIAM HITZ, of Polk City, Iowa; and H. W. SWARTZ, of Waukesha, Wis. will place the Holsteins.

The National Jersey Show will be passed upon by W. W. YAPP, of the Univ. of Illinois; and J. O. ROWE, Davis, Calif., will name the winners in the Milking Shorthorn competition.

The same judges will make the awards in each of these breeds at the Intern. Junior Show, open to 4-H and F.F.A. owners, which will be held on Oct. 8.

The open class judging of the Brown Swiss, Guernsey, and Jersey breeds, is scheduled for Oct. 9 and 10, and Ayrshires, Holsteins, and Milking Shorthorns for Oct. 11 and 12.

Second Symposium on Foreign Chemicals in Foods

The second Symposium on Foreign Chemicals in Foods, organized by the Netherlands Assoc. for Nutritional Research under the auspices of the Commission Intern. des Industries Agricoles and the Bureau Intern. Permanent de Chimie Analytique, will be held at Amsterdam July 9-11. All correspondence should be addressed to MISS E. W. WALLER, assistant secretary, Henri Polaklaan 42, Amsterdam (C).

Food Sanitarians to Meet

The 43rd annual meeting of Intern. Assoc. of Milk and Food Sanitarians will be held Sept. 5-7 at the Hotel Olympic, Seattle, Wash., according to an announcement from IAMFS president, H. S. ADAMS. In addition to hearing a large number of technical papers on many diverse matters in the field of milk and food technology, the convening sanitarians will see the presentation of the annual Sanitarian's Award, carrying with it a check for $1,000, to the municipal or county sanitarian who has made the most meritorious contribution to the health and welfare of his community in the past 5 years.

The committee planning the meeting is composed of: PAUL CORASH, president elect, chairman (New York City); H. B. ROBINSON, second vice-president (Washington, D. C.); H. H. WILKOWSKE, secretary-treasurer (Gainesville, Fla.); and C. S. ADAMS, Washington State Milk Sanitarians Assoc. (Olympia), chairman of the local arrangements committee.

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NEW VAC-HEAT PRODUCT TREATING PROCESS

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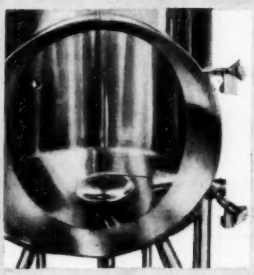

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The Whole Family Will Like the Double Protected Flavor of Your Milk. You Can Build Business by Advertising and Merchandising VAC-HEAT Processing

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News from Louisiana State

The first annual Louisiana Dairy conference was held at the State Univ. on Feb. 23 and 24. Staff members in dairying from several substations and those of the main station attended. As this meeting was the initial one of this nature, the addresses covered history, organization, physical features, and past research, as well as present work. An over-all state-wide program for the future with coordinated projects fitting into the whole scheme was favored by the group of workers.

C. W. UPP, director of resident instruction in the College of Agriculture, has been chosen director of the Agricultural Experiment Station, effective July 1, 1956. He will succeed J. N. EFFERSON, who will become dean of the College of Agriculture on that date. DEAN J. G. LEE, JR., will retire June 30.

MR. and MRS. JOHN KINCHEN of Sunshine, La., are the owners of Glover Valentine Rag Apple, a registered Holstein-Friesian that has set a new state record in Herd Improvement Registry. According to the Holstein-Friesian Assoc., this cow now leads all Louisiana Junior 3-year-old Holsteins milked twice daily in the H.I.R. test with a production of 12,162 lb. of milk and 512 lb. of fat.

E. B. MORGAN has accepted an appointment as farm supervisor of the State Univ. dairy farm. He received the B.S. degree in dairy production at L.S.U. in 1951 and the M.S. degree at the same institution in 1953. From that time until his appointment he served with the occupation forces in Korea.

Diversey Promotes Berst

L. SHERE, president of the Diversey Corp., Chicago, announced the promotion of N. W. BERST to Director of Research Laboratories. In his new position, Dr. Berst will coordinate and guide new product development, improvement of current products, and evaluations of competitive products. He joined the Diversey Corp. in 1951 as group leader in charge of organic research. In 1954 he was selected to attend the Oak Ridge Institute of Nuclear Studies with 31 other leading scientists.

Kerr Wins Promotion in C. P.

The Creamery Package Mfg. Co. has announced the promotion of H. J. KERR to the position of Director of Machinery Sales. Kerr joined the Company upon his graduation from Michigan State Univ. in 1930, gaining brief experience in the DeKalb factory before transferring to General Office Engineering and Research. Later, after serving as Sales Engineer for the Chicago Branch, he was promoted to Manager, in 1939, and has handled this assignment ever since, with the exception of the period from 1942 to 1945, when he was the Company's Director of War Projects.

Recent Mergers

Foremost Dairies, Inc., has recently acquired the H. A. MCDONALD Creamery Co. of Detroit; the Jones & Griest Ice Cream Co., Washington, Pa.; the Florida Juice, Inc., distributors of fresh citrus juice in the state of Florida and Metropolitan New York City; and the delivery system of the Purity Milk Farms, Fresno, Calif.

Borden Promotions

D. K. BASKERVILLE has been appointed sales manager of the Wisconsin Fluid Milk and Ice Cream Division of the Borden Co. at Milwaukee. To fill Mr. Baskerville's former position of general manager of Borden's Kennedy-Mansfield plant at Madison, HENRY SOLDWEDEL has been transferred from Borden's Pekin, Ill., plant.

Time to Control Flies

The fly season is just around the corner, and now is a good time to take the first step in any fly control program—sanitation. Clean up fly-breeding areas at this time to prevent a large population from developing later. Build-up in fly population will also be slower if the premises are kept clean. Clean out calf and bull pens and other areas where you find decaying organic material. Spread manure piles out to dry. Clean garbage pails and scrub them regularly. A solid bottom and tight-fitting cover on the garbage pail helps to prevent fly breeding.

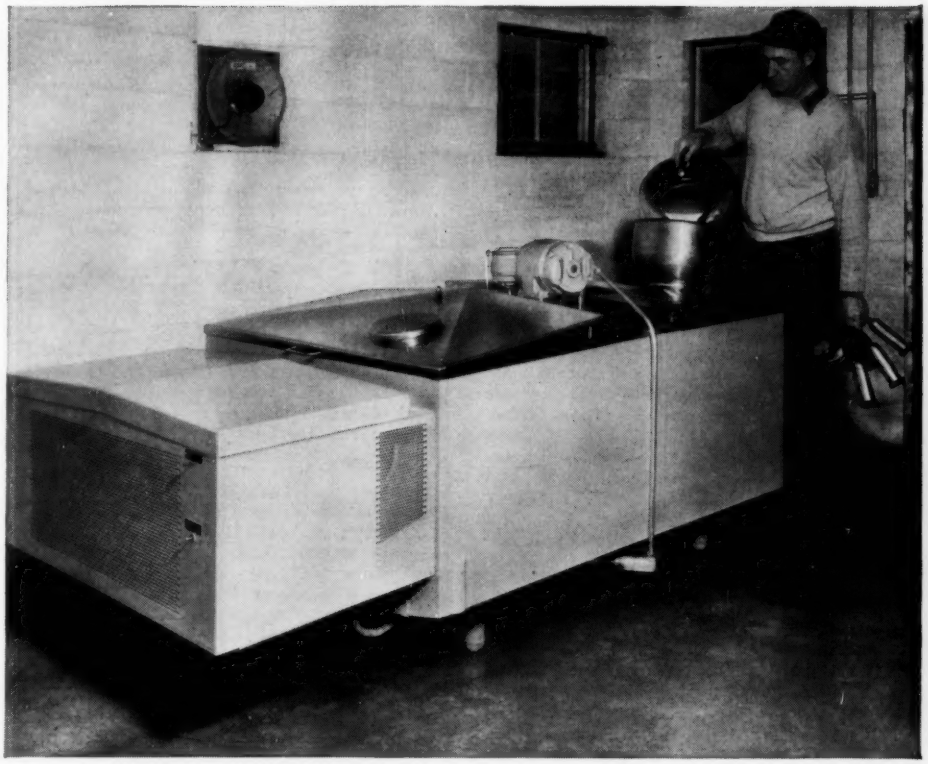
"No Smoking"

Careless smoking and misuse of matches are by far the leading causes of fire. Cultivate careful smoking habits. Do not smoke in the barn, in storerooms or near flammable liquids. Post "No Smoking" signs in appropriate places.

Accident Reduction

The safe operation of any machine depends entirely on the operator. You must always be alert, quick, and agile if you are to avoid some of the sudden emergencies that arise to cause serious accidents. To reduce these, learn what precautions to take with each machine, learn safe habits that are common to all machines and then practice them until they become automatic.

Mark significant events by making contributions to Dairy Remembrance Fund, 111 N. Canal St., Chicago 6, Illinois.



Why Jim Sugden swears by his new "Kold-Pak" farm tank

Let Jim Sugden, R.F.D. #2, Mukwonago, Wisconsin, tell you about his 200-gallon Cherry-Burrell "Kold-Pak" Farm Tank. He can do it better than any professional.

"Low pouring height is its biggest advantage. My wife feels it's the easiest-to-clean tank we have seen. It's so low. We were worried about milkstone build-up, but haven't had a bit of it.

"Your direct-connected refrigeration system was mighty easy to install and hasn't caused us a bit of trouble.

"Our milk hauler tells me this is the best draining tank on his route.

"We are glad we bought a tank with vinyl plastic finish because it doesn't water-spot—keeps its clean, new look.

"I'd buy another Kold-Pak." (The italics are ours. And thank you, Jim.)

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Recent Deaths

ROBERT S. BREED died Feb. 10 at his home in Geneva, N. Y., at the age of 78. He retired in 1947 after serving 34 years as professor of bacteriology. Dr. Breed was internationally known for his work in the bacteriology of cheese and the classification of bacteria. He had been editor-in-chief of *Bergey's Manual of Determinative Bacteriology* since 1937.

GERALD (GERRY) HEEBINK, 61, extension dairyman at West Virginia since 1935, died suddenly at his home in Morgantown Nov. 10. Mr. Heebink had participated widely in various phases of the dairy industry of the Mountain State, including supervision of D.H.I.A., the W. Va. Dairymen's Assoc. (of which he had been secretary since 1935), the W. Va. Dairy Cattle Show, Artificial Breeders Coop. of the state, the W. V. U. Dairy Short Course, and other dairy organizations. At the time of his death, he was serving as state chairman for June Dairy Month.

Born at Baldwin, Wis., and a veteran of World War I, Mr. Heebink earned his B.S. and M.S. degrees at the Univ. of Wisconsin. Previous to becoming Extension Dairyman in W. Va., he served in the same position at South Dakota State College for 12 years. He is survived by his widow and one son, David, a member of the faculty of Stanford Univ.

L. A. HIGGINS, a graduate of North Carolina State College in 1910 (M.S. 1912), died Dec. 17 at his home in Asheville, N. C. A former USDA employee, Mr. Higgins spent 43 years of his life in Mississippi working in the interest of dairy farmers. He led in organizing the Mississippi Artificial Breeders Assoc., assisted in setting up the All-American Junior Jersey Show and in organizing the Mississippi Dairy Products Assoc.

J. H. HOWARD, for years a prominent leader in the certified milk field, died Feb. 9 in Baltimore, aged 72. He was a former staff member at Johns Hopkins Medical School, and served as president of the American Society of Bacteriologists in 1931. A graduate of Illinois College at Jacksonville, he received his M.S. degree from the Univ. of Illinois and his Ph.D. from Harvard Medical School.

JULIUS K. MARQUARDT, 62, assistant director of the division of milk control in the New York State Department of Agriculture and Markets, died at his home at Geneva, N. Y., March 7. From 1917 to 1920 he taught dairying at Stanford Univ. and for a number of years was connected with the dairy products research program at the New York Experiment Station at Geneva.

ROSCOE E. LITTLE, who served for many years as secretary of the International Milk Dealers Assoc. (later known as the Milk Industry Foundation) died Feb. 1. Mr. Little was the Association's first secretary. He retired in 1946. He is survived by his widow, who lives at Villa Park, Ill., and three children.

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The growth and prosperity of the dairy industry depends upon quality dairy products and lower production costs.

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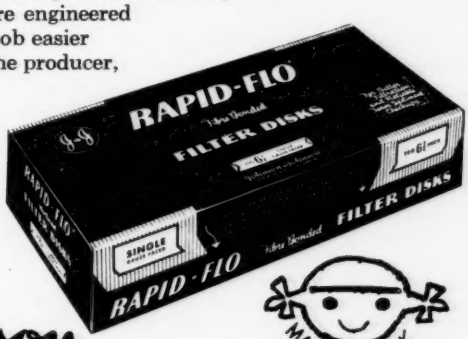
Your local Rapid-Flo representative will gladly help you with your program to improve quality and cut production costs.



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STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students

E. L. THOMAS, *Editor*

Outstanding Student Achievements

Top Dairy Products judge—1955. JOE A. SIMS was born Oct. 27, 1928, at Red Bay, Ala. When he was 4 years of age, his family moved to a dairy and cotton farm near Burnsville, Miss. Upon graduation from Holcut High School in 1947, he secured employment with the F.B.I. In September, 1948, he entered Northeast Mississippi Junior College and, after 1 year, entered the U.S. Air Force on Sept. 8, 1949. Joe received his discharge in February, 1953. While in the service he was a supply sergeant in charge of stock records in Tripoli, Liberia.

J. A. Sims

Sims entered Mississippi State College in the spring of 1954 and specialized in dairy manufacturing at the beginning of his junior year. At the end of the year, he received the Virginia Dare Award for flavor discrimination. He was a member of the Mississippi State dairy products judging team, which won first place in Milk and second place in All Products in the Southern contest at the Univ. of Missouri, and first place in All Products in the Collegiate Students' Intern. Contest at St. Louis in October, 1955. In this contest Joe was high man in All Products.

He is now an active member of the Mississippi Student Affiliate Chapter of A.D.S.A. and has headed various important committees of this organization. He will graduate in dairy manufacturing in May, 1956. He is particularly interested in sales. On October 18, 1952, he was married to Miss Joyce Maton of Pana, Ill.

Three Ohio State students honored. RONALD PERKINS and CHARLES BORROR, seniors in the Dept. of Dairy Technology, and GARY CRILE, a junior, have been awarded special honors during the winter quarter for their outstanding scholastic achievements and participation in student affairs.

Perkins, a native of Springfield, was awarded the R. B. Stoltz award in recognition of his qualities of leadership, scholarship, and interest in the dairy industry. He has served as president of the student dairy club, was a member of both judging teams that competed at the Midwestern and Intern. Judging Contests, and has supported himself financially throughout his college career. The R. B. Stoltz Award was established in 1945 to honor the late Profesor Stoltz. The income from a fund, deposited with the University, is used to award an annual cash scholarship to outstanding students in dairy technology.

Gary Crile, of Akron, was given the Boosters Award. Gary, who is married and the father of one child, has been completely self-supporting throughout his college career and has maintained a scholastic average well above a "B." He has served on the Dairy Technology Council and as chairman of the publicity committee of the Student Dairy Club. This award was established in 1945 by the Ohio Dairy Boosters Assoc. The income from the fund is used to provide a cash scholarship for a student in dairy technology at Ohio State Univ., who has demonstrated superior leadership qualities.

Charles Borror, of Columbus, was awarded the Virginia Dare Award for superior interest and performance in ice cream activities. Charles, like the other award recipients, has earned all of his college expenses, is married and the father of three children, and was listed on the Dean's Roll for high scholarship.

Georgia Chapter News

The programs of the Dairy Science Club at the Univ. of Georgia are composed primarily of guest talks, supplemented with movies and debates. Recently, J. D. GAY, manager of the Georgia Milk Producers Confederation, gave an interesting talk on the necessity of high quality management in today's dairy industry. During the month of January, the club heard BUBBER PIPPIN, a Georgia IFYE Exchange Student to Egypt. Bubber told the club of his experiences in Egypt and showed slides. Also, in January the club held a buffet dinner previous to the business meeting. The club will terminate the winter quarter by having a roll-call debate on the subject, "Bulk tank vs. canned milk pick-up for present day Georgia farms."

For the spring quarter, program chairman CHRIS NEWCOMER has in the planning stages a field trip to one of Georgia's certified dairies. Also, in the spring, the club has its annual Dairy Day, which this year will be held on April 21. It is sponsored for 4-H and FFA club members and leaders. DAVID BREESEN, Dairy Day chairman, is expecting a large attendance.

Illinois Club Honors Yapp and Rhode

The Dairy Production Club at their annual banquet honored retiring dairy faculty members, W. W. YAPP and C. S. (DUSTY) RHODE. Dr. Yapp will retire in September. He has

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been teaching at the Univ. of Illinois for 45 years. He is one of the best known judges of dairy cattle in the nation and has judged dairy cattle at 15 national and international dairy shows, in addition to more than 100 state fairs. Professor Rhode has served 39 years as an extension dairyman in Illinois. He was a pioneer in the field of cooperative artificial breeding. Rhode also established the Dairy Herd Improvement Association work at Illinois.

Kentucky Club Sponsors Showmanship Contest

Over 20 students of the Univ. of Kentucky competed in the Dairy Club's annual showmanship contest at the Dairy Center Arena, Jan. 19. R. D. GIBSON acted as M. C. and introduced the participants showing Jersey calves, yearling Jerseys, and Holstein calves. E. C. SCHEIDENHELM, Univ. of Kentucky extension dairyman, acted as judge.

A senior from Alexandria, Ky., JOHN WOESTE, was named Jersey champion, for which he received a trophy donated by the Kentucky Jersey Assoc. Mr. Woeste was also selected as grand champion showman and will have his name engraved on a plaque hanging in the hall of the Dairy Products Building. LEON DAVIS, a Russelville, Ky., sophomore, was selected as Holstein champion and received a trophy donated by the Kentucky Holstein Assoc.

Several girls competed in a milkmaid's contest, which was won by SHIRLEY FORD of the College of Agriculture and Home Economics. The U. K. Dairy Club furnished milk to the entire audience, and door prizes were also awarded.

Louisiana Team Competes

The Louisiana State Univ. dairy judging team, competing with six other schools, tied for first place with the Univ. of Arkansas at the Southwestern Exposition and Fat Stock Show Feb. 9, 1956. The team also took top honors in judging Holsteins and Guernseys. Last year the team led all competitors at the same show and went on to win fourth place at the National Dairy Cattle Congress in Waterloo, Iowa. The team was coached by J. B. FRYE, JR., head, L.S.U. Dairy Dept.

O.S.U. Club Activities

The Ohio State Univ. branch of A.D.S.A. served 441 lunches for the 23rd annual Dairy Technology Conference, held on the campus Feb. 7-11.

On Feb. 25, the annual dinner-dance was held in the Ohio Union. MENO LOVENSTEIN of the Dept. of Economics was the main speaker. The Honorary Member Award was presented to VERN CAHILL for his outstanding services to the student organization. Student awards were given by I. A. GOULD and FORDYCE ELY to DONALD RICHARDSON, RONALD PERKINS, CHARLES BORROR, and GARY CRILE. Judging keys were presented to members of the 1955 dairy cattle and dairy products judging teams.

The club's annual spring picnic will be held on May 20 at Holiday Hill, Delaware, Ohio.

OUR ASSOCIATION

Reading and Writing and Support

DEAR FELLOW MEMBERS:

An executive of our industry, who is president of his own progressive and rapidly expanding business, visited us recently and commented, "Too often the college graduates in my organization don't read. They are not members of the American Dairy Science Association—nor do they read the Journal and similar publications to keep abreast of research and scientific discoveries. The result is that they have stopped growing mentally and professionally and will not give me the type of leadership I need in my organization in future years."

This executive is educated as a dairy technologist, and his success in the industry has revealed the value of a technical background. His view recalls the maxim, "He who can read and does not read is no better than he who cannot read." T. KLINE HAMILTON, when president of the Milk Industry Foundation, ofttimes said, "When we are through improving, we are through!" Perhaps a person submits his own evidence of "being through" when he stops reading his professional and technical journals; when he fails to capture from them the new ideas as they are first revealed and tends to draw his daily conclusions on the basis of past education and past experiences which may become, from one day to the next, obsolete.

The statement made by this executive brings into focus several important points. For one thing, it accentuates the responsibility of those of us in education who must, in some way or another, bring to our undergraduate students the realization that their education is just beginning when they receive their degree. We need to stress the fact that their continued educational growth depends upon constant and maximum utilization of their mental capacities to read and study in their own field.

Another need is for our members to do a more thorough job in bringing to professional dairymen the real value of membership in our Association and thus obtain subscriptions to the Journal. The Journal is a technical, scientific, and professional publication and it is not purported to be a popular-style presentation. However, this does not warrant an apology but rather should be the basis for considerable pride. The Journal presents facts and research findings in a cold, unglamorized fashion—and from these facts and findings come worth-while ideas. In this day, science is a way of life—it is part of our daily experiences, and an educated and intelligent person is expected to be conversant with the common scientific language and the major research results in his own area.

Furthermore, it is not to be expected that the original research papers that appear in the Journal constitute "easy" reading. After all, this is a "professional" publication; it requires a thoughtful, analytical, and intelligent approach on the part of readers who are capable of sorting out very quickly the findings and ideas which may be applied to their specific activities. Even so, the Editor and his Board are constantly striving to make all of the articles as readable as possible, but there is a limit of readability for each scientific paper beyond which it is neither possible nor ethical to go.

A member of the Association and any other reader of the Journal should realistically accept the fact that not every article will be of great interest to him, and a particular issue may contain many articles that do not bear specifically on his needs. The Journal is attempting to cater to a wide variety of interests in the dairy field—interests which are as varied as the enterprises that comprise this large industry. Members of the Association include educators, scientists, administrators and executives, supervisors, technicians, salesmen, public health personnel, nutritionists, extension workers, public relations leaders, and others. Therefore, it is little wonder that not all aspects and phases of the industry are treated at any one time or in any one issue of the Journal. One can appreciate the tremendous problem which faces the Editor each month.

In respect to the matter of varied interests of the readers, the Journal is not dissimilar to the publications of other professional groups. Certainly, a journal in chemistry does not in every case meet the requirements of all chemists; neither does a journal in engineering satisfy at all times the widely different subject areas of this field. These professional journals, as that in our own industry, are to be accepted as a resource tool to be used as the material and occasion warrant.

The two main areas of subject matter in our Journal are dairy production and dairy manufacturing—and a reader may complain that there is too much emphasis on the phase in which he does not have specific interest. This complaint may be justified under certain conditions. However, the Editor is dependent upon active research workers for his supply of manuscripts, which he publishes in order of their receipt. Consequently, if a large number of production papers are received at one time, then it is likely that an issue of the Journal will be largely made up of articles in this field. The opposite situation also may exist. In recent months, the proportion of manufacturing to production papers has been approximately 4 to 6. Naturally, the Editor would like to obtain a more equitable balance.

In addition to a variety of scientific papers, the effectiveness and value of the Journal depend upon a steady supply of papers from institutions and organizations where research in dairying is being conducted. This immediately raises the question as to the relative creativeness and productivity of men who are ostensibly employed to conduct this research.

Recently, an experiment station director evaluated the work of his personnel on the basis of their publications and scientific contributions; the analysis of the results was revealing, if not startling. Perhaps this approach is justified in many of our educational institutions to make certain that the persons who are employed in research not only do the research but prepare the results immediately for publication.

Dr. N. P. Hudson, when dean of the Graduate School at The Ohio State University, frequently stated his philosophy that "no research is complete until it is written up." Perhaps this may be carried one step further by saying that "no research is complete until it is written up and published." When a person in the commercial field raises the question about the proportion of certain articles in our Journal, he might be directed to look to his own Land Grant college to find out how many articles that particular institution is publishing which deal with his area of interest.

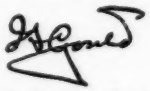
Perhaps every dairy department could do well to examine its records in order to determine the productivity of each research worker in respect to scientific publications. Certain research workers have commented that they are not interested in publishing but only in the research itself as it may benefit them and their knowledge. In such a case, the individual is a handicap to an institution or to a government agency, for the full value of the research, and the investment in it, can be achieved only if the findings are released as quickly as possible for general inspection and application.

A question relating to the support of the Association and the Journal was asked recently by a member of the Membership Committee when he said, "One of our main problems is to get members of our industrial organizations to join. Often one man in the company belongs —and the copy of the Journal is passed around for all of the college men to read. Thus, they get the advantage of the publication at no cost. What is the answer to this problem?"

There is no clear solution to this problem, although some scientific organizations have attempted to restrict circulation of certain publications to members only. However, it appears that as a matter of pride a person with proper qualifications would want to be a direct participant in the scientific and educational association in his field. He needs to realize that the financing of such an association is extremely difficult. It costs a great deal of money to produce the *Journal of Dairy Science*. In fact —if one would consider the total costs of all persons and facilities involved in conducting the research for each of the papers which are presented, as well as the actual cost of the Journal itself, he would have a better appreciation of the large monetary value of a single issue.

It seems logical that every graduate from a dairy school would feel a sense of obligation and loyalty to his professional organization and would want to contribute his small bit to help make certain that the group remains strong and influential. This he should accept as an obligation, whether or not he reads the Journal and whether or not it is, in his opinion, of any specific benefit to him. This is, in effect, the principle of supporting a "just cause"— where the benefits to the individual may not always be clear—but where the support makes possible the continuation of a worth-while industry program.

Cordially yours,



I. A. Gould, *President*

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Robert H. Rumler

Executive Secretary

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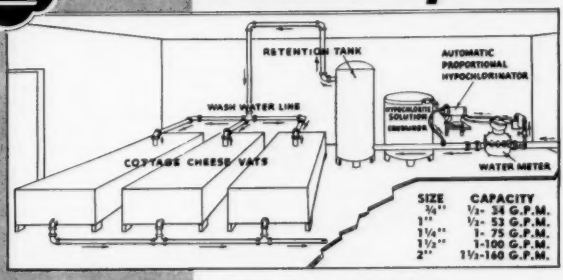

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ELECTROCHEMICAL MEASUREMENTS ON MILK WITH CATION AND ANION SENSITIVE MEMBRANE ELECTRODES[1]

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For many years there has been considerable interest in the ionic atmosphere of milk, its distribution into different phases, and its effect upon the stability of the colloidal systems in milk and on the keeping qualities of milk and of milk products.

The purpose of the investigation reported here was to determine if possible the normal over-all cationic and anionic activity of milk and to investigate the possible distribution of the various cations common to milk.

Three types of ion sensitive membrane electrodes have been used in this work and are as follows: Ion exchange resin membranes which are sensitive to all the cations present, clay membranes which are sensitive only to monovalent cations, and membranes prepared from anion exchange resins which are sensitive to all the anions. A previous paper by Affsprung *et al. (1)* reports on the preparation, characterization, and electrochemical behavior of the ion-exchange membrane electrodes that were used in this report. Membrane electrodes prepared from Amberlite IR-120-K resin were used for the determination of total cationic activities. The monovalent selective clay membranes were prepared from potassium montmorillonite (Wyoming benonite). The preparation and properties of this type of membrane have been described by Marshall and Bergman *(5)*. The measurements of anionic activities were made with Amberlite IRA-400-Cl membranes. These membranes were prepared and characterized in the same manner as the Amberlite IR-120-K membranes. The experimental procedure consisted simply of placing the skimmilk, or true solution sample, in one side of a concentration cell and a solution of potassium chloride of known activity in the other side of the cell. The potential difference across the membrane was then measured with matched calomel electrodes. The solutions, both reference and experimental, were changed at intervals of 5 to 10 minutes until the measured potential became constant. The time for equilibrium to be established was short; usually 10 minutes was sufficient for the potential to reach its

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Journal Series Paper No. 1475 approved by the Director of the Missouri Agricultural Experiment Station.

[2] The experimental data were taken from a thesis presented by H. E. Affsprung to the Graduate School, University of Missouri, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1954. Present address, New York State College for Teachers, Albany, N. Y.

final value. However, approximately 30 minutes of soaking and a minimum of three changes of reference and experimental solution were made before the final potential measurement was taken, except when the sample had been heated. For the heated samples only a 10- to 15-minute lapse of time occurred between cooling and the final potential measurement.

As the ion exchange membranes are sensitive to all the ions, monovalent and divalent, that occur in milk, generalized activities were calculated from the potential measurements on milk and defined as "monovalent equivalents." The calculations were made as follows:

$$\text{EMF} = \frac{0.059}{1} \log \frac{\text{experimental sample as ``activity of monovalent equivalents''}}{\text{activity of reference}}$$

Each membrane was held to a reproducibility of less than ± 0.5 mv., and if the membrane did not give values within this limit it was discarded. The membranes were also required to give an equilibrium potential that did not deviate more than ± 1 mv. from the theoretical potential when used in true solutions of potassium chloride of concentrations comparable to those in milk. The measurements were made with a Leeds and Northrup Type K-2 potentiometer at room temperature. As small changes in temperature do not markedly affect the measured potentials, it was not necessary to control the temperature. The use of such an empirical activity as defined above allows the comparison of different samples of milk when different reference solutions are used and also affords a means for the estimation of the distribution and nature of the various cations and anions contained in milk or processed milks.

The exact calculation for the activities of the individual ions cannot be made for a solution such as milk, which contains four different major cations. It is possible to calculate the individual ion activities in solutions containing two cations if the mobility ratio of the two ions within the membrane is known, and if the activity of one of the ions can be determined independently. McLean *(6)* has shown that when two different selective clay membranes in which the cations have sufficiently different mobility ratios were used in conjunction with a membrane that measures total activity, values for the activities of sodium and potassium in clay suspensions could be determined in a solution that also contained calcium ions. The calcium ion activity could then be approximated from the total cation potential obtained with a membrane sensitive to all the cations present. It was not possible to use such approximations in studies on milk, as the solution is more complex and a sufficient number of selective clay membranes was not available.

It has been found that the average potential obtained for milk samples with a group of four Amberlite IR-120-K membranes was very nearly equal to the average potential of any other group of four similar membranes. Thus, any variations in the mobility ratios of the cations in the individual membranes will be small as long as the major constituent of the experimental solution is the same as the ion in the reference standard solution. Also, the assumption would be

good that the mobility ratios for the different cations would be nearly equal in all membranes of the same resin prepared and characterized according to the method as described.

Potential measurements on raw skimmilk. A series of potential measurements were made on raw skimmilk by using general cation-sensitive membranes prepared from Amberlite IR-120-K, monovalent selective clay membranes prepared from potassium montmorillonite, and general anion-sensitive membranes prepared from Amberlite IRA-400-Cl. The data are presented in Tables 1 and 2.

TABLE 1

The potential and activities of various samples of raw skimmilk, measured by cationic and anionic membrane electrodes and selective clay membranes

Skimmilk sample No.	EMF and monovalent equivalents					
	Cationic	(a^+) [a]	Anionic	(a^-) [b]	Selective	(a^+) [c]
	(mv.)		*(mv.)*		*(mv.)*	
1	44.3	0.050	—	—	—	—
2[d]	16.9	0.058	—	—	—	—
3	41.9	0.045	—	—	—	—
4	42.8	0.048	21.7	0.021	—	—
5	44.2	0.050	30.3	0.029	—	—
6	44.9	0.052	32.8	0.032	—	—
7	43.7	0.049	30.6	0.030	—	—
8	42.3	0.047	30.6	0.030	32.7	0.032
9	39.9	0.043	29.5	0.029	—	—
10	42.7	0.048	23.6	0.023	34.1	0.034
11	42.7	0.048	31.4	0.031	—	—
12	42.5	0.047	29.0	0.028	33.8	0.034
13	42.0	0.046	—	—	34.4	0.035
14	42.4	0.047	24.2	0.023	34.6	0.035
15	42.6	0.048	30.7	0.030	35.4	0.036
16	42.5	0.047	28.2	0.028	35.5	0.036
17	42.7	0.048	30.5	0.030	—	—
18	39.1	0.042	24.4	0.023	34.8	0.035
19	39.7	0.043	30.0	0.029	34.0	0.034
20	40.8	0.044	29.9	0.029	35.1	0.035
21	40.8	0.044	27.2	0.026	—	—
Av.	42.2	0.047	28.5	0.028	34.4	0.035
Standard deviation	±1.50		±3.07		±0.79	

Reference solution, a_{KCl} in all cases unless otherwise indicated = 0.009.

[a] Total cationic activity as "monovalent equivalents."
[b] Total anionic activity as "monovalent equivalents."
[c] Selective "monovalent activity" of monovalent cations.
[d] Reference solution, $a_{KCl} = 0.027$.

Each sample number in column 1 of these tables represents a different original sample of raw skimmilk obtained on a different day. The potential measurements on the skimmilk samples were made with two different activities of potassium chloride as the reference solution: $a_{K^+} = 0.009$ (Table 1) and $a_{K^+} = 0.027$ (Table 2). Each potential value listed in these two tables is an average of a minimum of four equilibrium measurements, one value from each of four different membranes. Both sets of measurements made with two different reference solutions gave an average calculated cationic "activity of monovalent equiva-

TABLE 2

The potential of raw skimmilk measured with Amberlite IR-120-K membranes

Skimmilk sample No.	EMF	Skimmilk sample No.	EMF
	(mv.)		*(mv.)*
1	15.1	9	15.0
2	14.6	10	15.0
3	14.6	11	13.8
4	14.9	12	13.7
5	14.5	13	14.0
6	14.6	14	13.6
7	14.8	15	13.6
8	14.9	16	14.4

Av. EMF = 14.4, standard dev. ± 0.52.
Calculated total cationic activity as "monovalent equivalents" = 0.047.
Reference solution, a_{KCl} in all cases = 0.027.

lents" of 0.047. The fact that the average calculated "monovalent activities" are the same when two different reference solutions are used lends support to the relative correctness of the numerical values and to the validity of the measurements. Also, the magnitude of the experimental potentials obtained from skimmilk was in the range where the membranes agreed closely with the theoretical Nernst potential. Thus, no corrections were necessary *(1)*.

A standard deviation was computed for each of the four equilibrium cationic potential values obtained from each of the 21 skimmilk samples listed in Table 1. An average standard deviation of ±0.64 mv. was then obtained from the 21 individual standard deviations for total cationic potentials. This standard deviation gives a measure of the reproducibility of the membranes when used with the milk samples. Also, standard deviations are given in Tables 1 and 2 showing variations of potentials from the mean for different milk samples.

In other experiments, samples of the raw skimmilk were subjected to various heat treatments; the most severe heating was 120° C. for 15 minutes. The milk was then cooled in running tap water, and the potential was immediately measured. In no case was there a change in either the total cationic or anionic potential between the heated and the unheated milks that was greater than the experimental error. The results would indicate that either the over-all activity changes are small or the ionic equilibrium of milk compensates for some insolubilization of the calcium and phosphate, the latter being the most likely. The heat treatments used did not cause a change in the pH of milk of sufficient magnitude to affect the membrane potentials. The greatest change in pH was a drop from 6.76 to 6.48.

Membrane potential measurements on a quaternary solution of the cations common to milk. A ternary matrix solution was prepared containing potassium, sodium, and magnesium chlorides. Different amounts of calcium chloride were added to this ternary solution in order to determine the minimum detectable change in the activity of calcium chloride as measured by resin membrane electrodes. When the ternary matrix solution was diluted with an equal volume of water, the final concentration of the sodium, potassium, and magnesium ions in the solution was of the same order as is normally found in milk. To this solution,

before dilution, known amounts of calcium chloride were added, the solution was diluted, and the potentials were measured. The data are given in Table 3.

The numerical potential values listed in each column of Table 3 for the six Amberlite IR-120-K membranes are not directly comparable because in mixtures of ions each membrane is unique and its behavior is a function of the internal structure and charge of that membrane. Each potential value is for a single

TABLE 3

Change in EMF of a quaternary solution with added calcium chloride (EMF in millivolts)

Membrane No.	Equivalent concentration of added calcium chloride[a]					Measured Δ EMF	Calculated Δ EMF
	0.0	0.001	0.003	0.005	0.010		
1	44.2	44.0	44.3	45.2	46.4	2.2	2.7
2	41.2	42.1	42.2	42.6	43.1	1.9	3.0
3	47.0	46.1	47.7	49.1	50.7	3.7	3.9
4	46.7	46.6	47.9	48.9	50.0	3.3	3.5
5	43.6	44.7	45.1	46.2	47.3	3.7	3.2
6	42.9	44.1	44.3	45.3	46.3	3.4	3.0
Av.	44.2	44.6	45.3	46.2	47.3	3.1	3.1
Standard deviation	±2.25	±1.62	±2.22	±2.46	±2.77		

Av. standard deviation ±2.26.
Reference solution, a_{KCl} in all cases = 0.009.

[a] $CaCl_2$ added to a ternary solution of potassium, sodium, and magnesium as the chlorides of 40 meq/l of K^+, 22 meq/l of Na^+, and 9 meq/l of Mg^{++}, respectively. The total concentration of cations in the diluted ternary matrix solution was 0.071 eq/l. The final quaternary solution containing the added $CaCl_2$ of 0.01 eq/l had a total concentration of 0.081 eq/l.

equilibrium potential measurement with a particular membrane, and each membrane was used over the entire concentration range. It was found, however, that any given set of four membranes will give an average value nearly equal to that of another set. It will be seen upon considering the potential values given in Table 3 that an observable increase in potential was not obtained until the equivalent concentration of added calcium was 0.005 to 0.01. However, a gradual increase occurred in the average potentials from 44.2 to 47.3 mv. over the added concentration range. These values marked the lower limits of concentration change that could be detected and are near the 10 mg. % level of calcium in milk. The values in Table 3 also show that the average change in potential obtained with several membrane measurements for quaternary solutions containing added calcium chloride falls close to that calculated from the simple Nernst equation for concentration cells. The probability that the difference in average potential between the matrix solution alone and the matrix with 0.01 eq/l of added calcium chloride is significant is 0.9 when only the last four membrane measurements are considered. The measured change in potential between the matrix and final solution as determined by the first two membranes is considerably less than the others, the measured Δ EMF being 2.2 and 1.9 mv., respectively. It is possible that the mobility ratios of calcium ion and the other ions are too great for reasonably accurate measurements with this pair of membranes. Nevertheless, remarkably good agreement is observed between the average experimental and calculated differences in potential.

The over-all cationic concentration of the diluted ternary matrix solution was 0.071 eq/l and the average cationic potential was 44.2 mv., a value close to that obtained for milk (Table 1). The calculated activity of the matrix solution from the Nernst equation, made with the average cationic potential of 44.2 mv. was 0.051 eq/l. This gives a generalized activity coefficient of 0.72 for the ternary matrix solution. The value of 0.72 for the activity coefficient for this ternary solution is in good agreement with values calculated from the Debye-Huckel theory for solutions of the same ionic strength and with reported values of activity coefficients for solutions of the same salts in the same concentration ranges *(4)*. In order to obtain the calculated potentials of the quaternary solutions to which 0.01 eq/l of calcium chloride was added, an activity value of 0.006 was added to the initially measured activity of the ternary matrix solution (0.051) to give the final activity of the quaternary solution (Table 3). The activity coefficient of 0.6 was obtained by the use of the Debye-Huckel approximation for aqueous solutions, and it is in accord with measured values of the activity coefficient for calcium chloride in solutions of the same ionic strength *(7)*. The average ternary matrix solution potential of 44.2 mv. was subtracted from the calculated potentials for the quaternary solutions containing 0.01 eq/l of added calcium chloride. The differences in potential are tabulated in the last column of Table 3 as "calculated Δ EMF."

The average measured potential for the final quaternary solution containing the added 0.01 eq/l of calcium chloride was 47.3 mv. As the total amount of calcium ion was small compared to the other cations present, the generalized activity coefficient of 0.72 was used to calculate the final "concentration expressed as monovalent equivalents" for the matrix solution containing 0.01 eq/l added calcium chloride. The calculated value for the total concentration of "monovalent equivalents" from the experimental value of 47.3 mv. was 0.082 eq/l, whereas the actual value was 0.081 eq/l. These data agree very well and support the use of the value of 0.72 as a generalized activity coefficient for the calculation of the concentration of "monovalent equivalents" in milk samples. However, this value is probably somewhat high since milk contains proteins, sugars, and other substances that would reduce somewhat the activity of ions in milk.

Upon applying the activity coefficient of 0.72 to the data in Tables 1 and 2, the total concentration of cations in milk was found to be 0.065 eq/l, and that of the monovalent cations as obtained from the selective clay membranes to be 0.049 eq/l. The total anion concentration was 0.04 eq/l. The average literature values for the concentration of potassium and sodium are 0.04 and 0.022 eq/l, and that for the chloride is 0.03 eq/l *(8)*. The literature values given for total K^+, Na^+, and Mg^{++} are close to the averages found for Missouri milk. It was not considered necessary to assay each milk sample on which membrane measurements were made.

The difference between the total cationic concentration and the monovalent concentration only as determined by membrane measurement was 0.016 eq/l. If the average literature value for magnesium of 0.009 eq/l *(8)* is subtracted from the value 0.016, the difference 0.007 eq/l represents approximately 14 mg. % of

the calcium as being in the ionic form. This would be a minimum figure for ionic calcium and assumes that the total magnesium content of milk would exert an activity effect. As the effect of the mobility ratios upon the measured potentials lowers them below the Nernst value, and as the activity coefficient of 0.72 is probably too large for the divalent ions, this value certainly represents a minimum for the ionic calcium concentration in milk. Also, it has been reported *(8)* that approximately 70% of the total magnesium present in milk is in true solution; thus, the ionic concentration of magnesium in milk is closer to 0.006 than to 0.009 eq/l. When this fact is taken into consideration, the ionic calcium content of milk calculated from membrane potential data is 20 mg. %.

In another approach to the problem, the concentration of ionic calcium in skimmilk has been determined by an independent method developed in the Missouri laboratories, which is called the Resin Contact-Time Method *(2)*. An average value of 23.8 mg. % was found for ionic calcium in a series of five different samples of skimmilk. These data confirm the values obtained from membrane electrode experiments. In these experiments the ionic concentration of calcium was directly determined.

The difference of +0.01 eq/l between the measured anion concentration and the reported concentration of chloride was probably contributed by phosphate and some by citrate.

Potential measurements on raw skimmilk after the addition of salts common to milk. To study the possibility of interactions between potassium, sodium, and calcium ions with the other constituents of milk, a series of solutions of increasing concentrations of the chloride salts of these cations was made in raw skimmilk, and the corresponding increases in potential were measured. The solutions of potassium and sodium chloride in milk were prepared by dissolving the weighed dried salt in raw skimmilk and diluting with milk to the desired volume. For the solutions of calcium chloride in milk a solution of calcium chloride of approximately one molar was prepared and standardized. Measured amounts of this standard solution were added to raw skimmilk from a semi-micro buret. The chloride salts were added to the milk in amounts to give solutions with a final concentration ranging from 0.02 to 0.20 eq/l. The changes in potential were measured with Amberlite IR-120-K membranes with a potassium chloride solution of known activity as the reference solution. All measurements were made at room temperature, and the membranes were checked with standard solutions at the beginning of, during, and at the end of each series of measurements.

The potential data on raw skimmilk containing the added sodium, potassium, and calcium chlorides are given in Figures 1 through 3. Each plotted point on the experimental curves shown in these figures represents an average potential value obtained from 16 different measurements—four measurements from each of four different membranes. The theoretical curves shown on these figures were obtained as follows. It was assumed that the activity coefficients of the milk-salt solutions dropped from approximately 0.7 to 0.6 when sodium and potassium chlorides were added and from 0.7 to 0.5 on adding the calcium chloride. The changes in activity coefficients were adopted from data given by Latimer *(4)*.

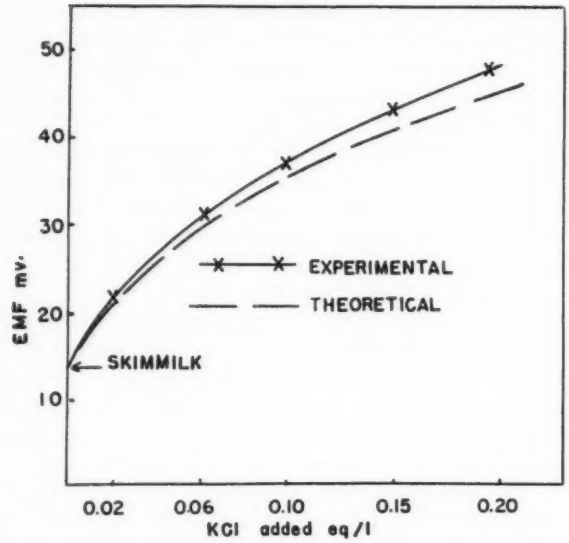

FIG. 1. The change in the EMF of raw skimmilk upon the addition of KCl. Activity of the reference solution of KCl was 0.027.

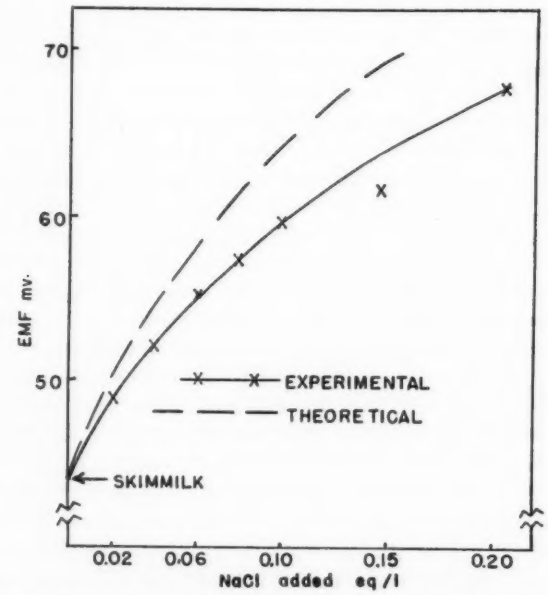

FIG. 2. The change in the EMF of raw skimmilk upon the addition of NaCl. Activity of the reference solution of KCl was 0.009.

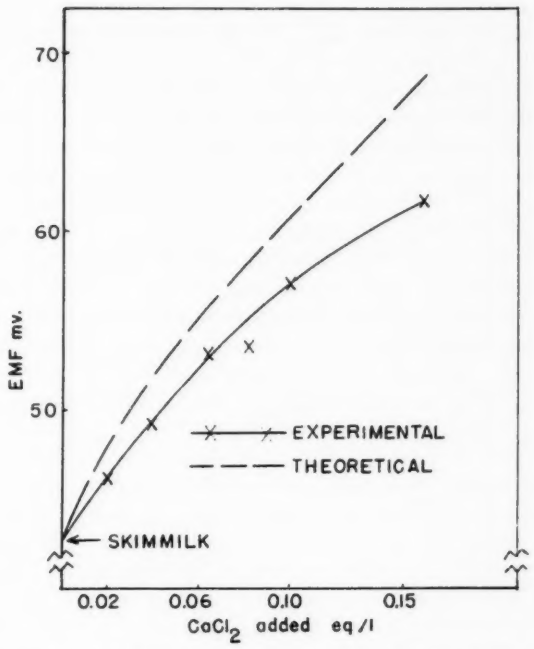

FIG. 3. The change in the EMF of raw skimmilk upon the addition of $CaCl_2$. Activity of reference solution of KCl was 0.009.

The activity coefficients that were used for the milk-salt solutions were obtained by plotting the literature values of activity coefficients for true solutions against the concentration and then reading the desired activity coefficient from the resulting curve. The activity coefficients obtained in this manner were used to convert the added concentrations (eq/l) of the respective salts to activities. These activity values were added to the activity of milk calculated from electromotive force data obtained by membrane electrode measurement. The calculated EMF for this final computed activity for each added concentration is a point on the theoretical curve. The assumed activity coefficients for the potassium chloride solutions in milk were somewhat low, but the agreement of the experimental with the theoretical curve was good (Figure 1). In the milks to which sodium and calcium chloride were added the assumed activity coefficients were too large and resulted in considerably greater theoretical potentials than the experimental (Figures 2 and 3). These deviations were more pronounced as the added concentrations of sodium and calcium chloride increased. In all of these measurements the reference ion was potassium. As the activity of the sodium and calcium ions in the milk solutions was increased relative to the potassium ion activity, a greater divergence of the experimental potential from the calculated theoretical potential obtained by adding activities resulted. When the per-

centage of an ion, other than the reference ion, in a solution becomes large, the response of the membrane does not follow the Nernst equation very well, as the mobility ratios for the various ions become important. The exact values for the different individual cationic or anionic activities in a solution as complex as milk cannot be calculated from the measured potentials. However, the total cationic, anionic, and selective membrane potential measurements on milk yield much useful information regarding the activities of the respective milk components.

As may be seen from Figures 1 through 3, in milk itself the concentration of monovalent equivalents calculated from averaged measured potentials cannot differ more than 10 to 15% from the true values and probably is closer when the reference ion is potasium, especially in view of the fact that the greater part of the measured cationic potential of milk is due to the potassium ion. The experimental potentials obtained for the smallest additions of potassium, sodium, and calcium chlorides (0.02 eq/l) agreed very well with the theoretical potential values. Also, the experimental and theoretical potentials for the milk-salt solutions containing added potassium chloride were in good agreement for the entire concentration range (0.02 to 0.20 eq/l). If the added ions form an insoluble or undissociated compound with a milk constituent, then the experimental potential curves for the addition of the various salts to milk should have a considerably smaller initial slope than predicted. This would certainly be true if the equilibrium activity product had been exceeded. The activity product could be defined by a $K_{eq.}$, which would be similar to a $K_{s.p.}$ or an instability constant. If the activity product had not been exceeded with the first additions of the salts, the experimental curve would follow the theoretical curve, and when it was exceeded, a break in the potential-concentration curve would occur. Neither a break in the curve nor an initial slope smaller than would be expected from the effect of mobility ratios was observed; thus, the only type of cation-protein binding would be that due to the formation of a double electrical layer with a resultant drop in activity. If chemical interaction had occurred, the initial slope probably would have been very nearly zero. Carr *(3)* reported on studies of this kind.

SUMMARY

This paper reports on the application of ion exchange resin membrane electrodes to studies on milk. Total cationic activities in milk were measured with membranes prepared from the resin Amberlite IR-120-K, selective clay membranes prepared from potassium montmorillonite were used to measure monovalent activities, and the total anionic activities were measured with Amberlite IRA-400-Cl membranes. Data are presented on the reproducibility of selected membranes, and an average standard deviation of ± 0.64 mv. was obtained for membranes when used in measuring the total cationic potential of a series of milk samples.

The total cationic activity of milk expressed as an "activity of monovalent equivalents" was calculated to be 0.047. The total anionic activity of milk expressed similarly was 0.028, and the monovalent activity of K^+ and Na^+ ions

as measured with selective clay membranes was 0.035. A "generalized activity coefficient" for milk of 0.72 was calculated from potential measurement data on a ternary solution containing potassium, sodium, and magnesium chlorides.

The concentration of ionic calcium in milk has been calculated from total cationic and selective potential measurements and is a minimum of 14 mg. %. When appropriate corrections are made for the magnesium content, values of at least 20 mg. % are obtained.

There were no significant differences in either the total cationic or anionic potentials for heated, as compared to raw, skimmilk. The results would indicate that either the changes are small or the ionic equilibrium of milk compensates for some insolubilization of calcium phosphate.

Membrane potential measurements were made on raw skimmilk after the addition of salts common to milk. The chloride salts of potassium, sodium, and calcium were added in levels ranging from 0.02 to 0.20 eq/l. There was no apparent chelation or chemical reaction of these salts with the milk components. The only type of cation-protein binding would be that due to the formation of a double electrical layer with a resultant drop in activity. The agreement of the theoretical and experimental potentials was good for potassium and fair for the sodium and calcium additions. It is also possible that some of the difference between the theoretical and experimental curves for the calcium additions to milk was caused by the formation of phosphate on citrate compounds.

Membrane potential measurements on milk and processed milks hold promise of yielding much valuable data on activities of milk components and how they change under certain conditions.

ACKNOWLEDGMENT

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SOME PHYSICAL PROPERTIES OF MILK. II. EFFECTS OF AGE UPON THE VISCOSITY OF PASTEURIZED WHOLE MILK[1]

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Numerous investigators have studied the viscosity of milk. It is generally agreed that its viscosity changes as it is held in storage at refrigerator temperatures *(1, 2, 5, 6, 13, 15, 16)*. The rate and extent of viscosity changes of milk as influenced by age have not been investigated critically.

During a study of the effect of homogenization of milk upon its physical properties, viscosity measurements were included. Many differences in milk samples (e.g., age and heat treatment before pasteurization, homogenization, and composition) may be expected to significantly influence several properties of the milk (e.g., density and the effect of age on the viscosity of the pasteurized milk). It soon became apparent that before such factors as homogenization pressure could be studied critically, more information was needed regarding the effect of age or time of storage upon viscosity. This paper describes a modified Ostwald viscometer and reports some effects of aging milk upon its viscosity.

Previous workers studying factors affecting the viscosity of milk did various things to avoid the complicating effects of age or time of storage on the viscosity of milk. Some investigators tried to avoid changes in milk caused by bacterial growth and enzymes. Others were more concerned with the effects of fat globule clumping, protein adsorption, and other physical changes. Taylor *(16)* determined viscosities within 4 hours after milking to avoid any changes in the milk caused by bacterial growth and enzymes. Tapernoux and Vuillaume *(15)* kept milk at 1° C. until it was examined 12 to 24 hours later. Bateman and Sharp *(1)* considered the effect age had upon fat globule clumping and viscosity. Caffyn *(2)* studied effects of temperature and attempted to eliminate the effects of age by measuring viscosities first at increasing temperatures and then at decreasing temperatures. Several investigators *(5, 6, 7, 9, 12, 13)* may have reduced the viscosity of aged milk and skimmilk to its original viscosity by heating and subsequent cooling.

Although aging effects are partially compensated by such methods, it seemed desirable to find a better adjustment or correction for changes in the viscosity of milk during storage when factors other than age are to be studied.

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PROCEDURE

The viscosity of milk, as measured in an Ostwald viscometer, varies with the driving pressure or head because milk is a non-Newtonian liquid *(1, 13)*. Variations in measured viscosity caused by changes in driving pressure were held to a minimum by making measurements at only low pressures. Further constancy was obtained by increasing the diameter of the capillary tube over that sometimes used *(1, 14)*. This also tended to eliminate irregularities in flow time caused by clumping of fat globules.

The slightly modified Ostwald viscometers used in this study are shown in Figure 1. The upper bulb was the double cone type with the common axis of the cones at an angle of about 40° to the capillary. This type of structure provided good drainage of the bulb with the capillary either vertical or inclined to as much as 70° from vertical. The lower bulb was made from a 25-ml. Erlenmeyer flask. The viscometer, which was placed in a water bath, was mounted in a steel channel support that would adjust itself to an exact position in the bath. The length of each capillary was approximately 72 cm. Diameters in different instruments varied from about 0.6 mm. to about 1.1 mm. Volumes of upper bulbs ranged approximately from 6 ml. to 7.5 ml. The bath had a volume of about 10 l. It consisted of a copper tube 4 in. in diameter and 30 in. long, with an inverted, wide-mouth, clear glass jar mounted on the top and open end. Part of the bottom was cut from the glass jar so that the viscometer could be inserted and the upper bulb covered by water when the viscometer was either vertical or inclined. All of the copper tube except about 2 in. at the bottom was insulated with asbestos pipe covering. To control the bath temperature, a gas burner supplied heat, and a stream of cold water or brine directed against the bottom of the bath served to remove heat. The bath was stirred by a stream of air bub-

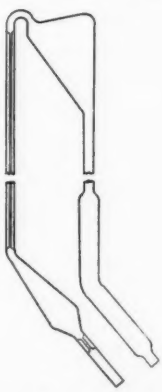

FIG. 1. Modified Ostwald Viscometer. In various constructions the length of capillary tube was about 72 cm. Its diameter ranged from about 0.6 mm. to about 1.1 mm. The upper bulb ranged in volume from 6 ml. to 7.5 ml. The lower bulb volume was about 25 ml. A mark 15 ml. from the open end of the large arm permitted use of this arm as a pipet for filling the instrument.

bles. Temperatures of water in the bath were observed to 0.01° C. with Beckman differential thermometers.

Stirring the sample by untimed flows helped attain temperature equilibrium. Gently forcing air from a rubber bulb down the capillary and through the sample also tended to decrease the separation of cream when whole milk was in the viscometer. Absence of drift in successive flow times was used as evidence of temperature equilibrium between the milk sample and the water bath. The flow times ranged from near 130 seconds at 4° C. to about 30 seconds at 44° C.

The flow times of the viscometers were measured manually with stop watches. The watches were type A-8 of U.S. Ordnance Spec. No. 94-27749, A.M. #3. Each watch was compared with another as follows: One was completely wound and allowed to run 2 minutes before each trial of a series, and the other was not wound during the entire series. Each watch ran slightly slow for about 1 minute after winding and slightly fast after about 1 hour. Within this interval no irregularity could be detected. When one watch ran continuously while its 10-second sweep was timed with the other, the standard error of timing was about 0.02 second. At times, when two viscometers were used for 3 or 4 days continuously, as many as eight observers were involved in a series of experiments. Before such service each operator demonstrated his ability to compare watches with this precision and to read flow times within 0.05 second of other operators.

Each viscometer was assigned its own stop watch and was calibrated against water. The value 1.0020 cps. for water at 20° C. *(14)* was taken as primary standard. The equation of Cragoe as cited by Coe and Godfrey *(4)* was used to calculate standards for comparison at other temperatures.

The precision with which flow times could be determined was studied as follows. A sample of milk was divided into 12 portions. Each of 11 portions was homogenized at one of the following homogenization pressures: 50, 100, 175, 250, 500, 1,500, 2,000, 2,500, 3,000, 3,500 p.s.i. One portion was not homogenized. The purpose of homogenization was to determine its effect on viscosity and to reduce creaming effects. After homogenization, the samples were cooled for a 5-minute period in an ice water bath to 4° C. and held at that temperature for 19 to 55 hours until viscosities were determined. Four measurements of time and temperature on a single sample, which had been adjusted to bath temperature as previously described, were averaged in determining each flow time used to calculate a viscosity. The average time was interpolated to exactly the desired temperature. The viscosities of each portion were determined at the following eight temperatures: 4, 8, 18, 22.7, 29, 34, 39, and 44° C. Therefore, 96 viscosity measurements were made in a trial as described above. Three such trials were made over a period of 4 months. For short flow times at 44° C., errors in measurement of time might be expected to have a relatively large effect compared with longer flow times at 4° C. The deviations of individual flow times in each set of four, from the average of these four, were used to give an estimate of standard deviation. This estimate includes effects of any undetected variations in the temperatures of the bath and the sample. The value thus obtained from 12 portions of each of three samples of milk was 0.0900 second, or a coefficient

of variation of 0.256% of the average flow time. This is equivalent to 0.045 second, or 0.128% for a mean of four trials. For the longest flow times at 4° C., the errors of timing were less important, and deviations calculated as above gave a better measure of the degree to which constant conditions of flow were maintained. The corresponding values were 0.1838 second, or 0.160% for an individual determination, and 0.0900 second, or 0.080% for a group of four. The larger deviations at 4° C. than at 44° C. indicate that not all the error was in starting and stopping the watch. The lower coefficients of variation at 4° C. indicate that the variations were not factors of flow time, i.e., were not differences, either in viscosity or in the viscometer, which were permanent during a complete flow time.

The effect of age upon the viscosity of milk was first studied with commercially homogenized milk. A fresh sample of mixed herd milk was pasteurized at 62° C. for 30 minutes and homogenized at that temperature in a Cherry-Burrell Viscolizer at 2,500 p.s.i. The milk was stored at 4° C., and viscosities were determined on aliquots at this temperature at 17 ages varying from 20 minutes to 300 hours.

In a second series of experiments the range of homogenization pressures and of temperatures at which viscosities were measured was extended. Mixed herd milk containing 4.0 ± 0.05% fat and 13.0 ± 0.10% total solids was pasteurized at 62° C. for 30 minutes. Portions were homogenized at 59° C. at seven pressures ranging from 100 to 3,000 p.s.i. One portion was not homogenized.

Pressures of 300 p.s.i. and less were obtained with a laboratory homogenizer *(11)* and pressures of more than 300 p.s.i. were obtained with a 125 gal. per hour commercial Manton-Gaulin homogenizer. After homogenization, the samples were cooled as previously described and stored at 4° C. until viscosities were determined. Samples in which extensive creaming was expected were divided into aliquots as soon as they had cooled to 4° C. Samples in which homogenization was more effective were mixed without warming whenever an aliquot was taken for viscosity measurements. Each aliquot was discarded after removal from a viscometer. Viscosities of each milk sample were measured at eight ages ranging from 10 to 300 hours. At each age the viscosity of each sample was measured at eight temperatures ranging from 4° to 44° C. Consequently, 512 viscosity determinations were made in replicates of four. Slopes *(b)* of the equation $\eta = a + b \log \text{age}$ were calculated by least squares *(8)* for both the 17 ages of commercially homogenized milk and the eight ages of each of the eight parts of the milks homogenized at various pressures. In this equation η = viscosity in poises $\times 10^5$, age = time in hours from cooling the pasteurized milk to measuring of viscosity, a = the fitted viscosity when log age = 0 (i.e., age = 1 hr.), and b = the slope of the regression line.

Densities at each temperature were measured as described in Paper I of this series *(11)*.

RESULTS

The effects of age upon the viscosity of pasteurized whole milk are shown in Figure 2 and Table 1. The data in Figure 2 were obtained from milk commer-

cially homogenized at 2,500 p.s.i. In this figure the straight line was drawn by the method of least squares. It is apparent that the viscosity of these samples of milk was approximately a linear function of the logarithm of the time of storage.

The data in Table 1 show a similar effect of age upon the viscosity of milk homogenized at various pressures ranging from 0 to 3,000 p.s.i. Each of the 64 values in the table representing slopes b was obtained, as for Figure 2, by fitting a straight line by the method of least squares to measurements of viscosity at eight known ages. The slopes ranged from 2.01 to 210.93, depending primarily upon the temperature at which viscosity was measured.

DISCUSSION

The approximate fit of the line to the circles in Figure 2 suggests the equation $\eta = a + b \log \text{age}$. This is the most satisfactory equation yet found. The slopes in Table 1 are used to adjust measured viscosities for age of sample.

Undoubtedly, some factors affecting changes in viscosity as a result of age are not included in this equation. For example, some points in the scatter diagrams (Figure 2 and Table 1) deviate from the regression line by several times the standard error of laboratory measurement. Furthermore, slopes of regression lines *(b)* at low temperatures appear to increase with homogenization pressure,

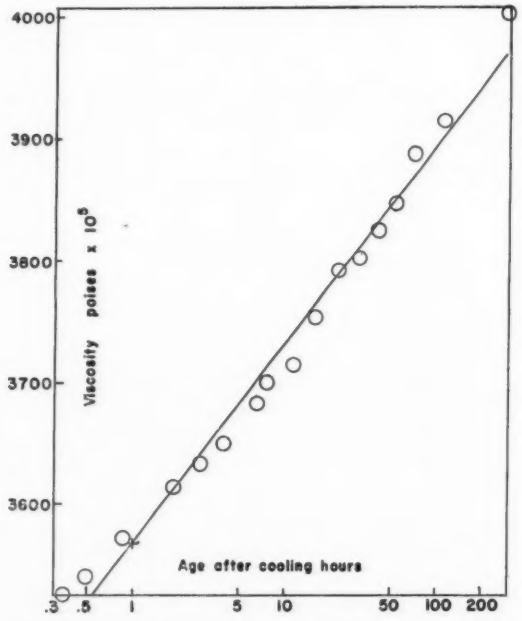

FIG. 2. Changes in viscosity of homogenized milk with age. Stored and measured at 4° C. Circles show measured values. Line is fitted by least squares through point + with slope 163.1.

TABLE 1

Slopes of straight lines fitted by the method of least squares to the equation, viscosity of milk = a + (slope) (log age)

Homogenizing pressure	Slopes, viscosity (poises × 10^5/units log age in hours at temperatures shown)							
	Temperatures at which viscosities were measured (° C.)							
	4	8	18	22.7	29	34	39	44
(p.s.i.)	Slopes of regression lines and their standard errors							
0	162.02	139.68	93.93	89.73	52.62	31.00	13.95	11.80
	±17.74	10.18	7.13	5.23	12.62	8.93	7.73	6.85
100	167.10	151.14	109.34	92.34	61.86	35.67	20.30	8.16
	±22.66	19.86	14.58	9.58	7.00	6.97	6.62	7.04
200	165.87	150.04	118.99	96.54	60.67	36.79	3.40	2.01
	±11.82	8.92	5.52	7.60	11.38	11.75	10.56	9.33
300	167.45	152.19	123.62	110.72	73.57	45.54	26.16	10.96
	± 7.86	9.11	8.64	8.16	10.89	8.69	6.85	4.31
500	186.37	150.56	99.06	88.07	40.69	28.31	14.29	2.56
	±12.58	13.24	10.05	8.77	9.24	5.17	5.34	4.57
1,000	198.19	162.84	105.30	80.73	57.59	34.77	18.97	14.07
	±13.47	12.75	7.09	9.46	7.67	5.14	4.20	3.73
2,000	201.61	169.16	122.31	96.65	58.91	41.13	27.57	14.61
	±21.63	12.21	5.15	8.29	5.98	5.37	3.23	2.43
3,000	210.93	162.35	121.94	99.42	63.34	40.78	22.91	15.51
	±16.26	12.75	7.43	7.15	7.40	5.09	4.79	2.68
Av.	182	155	112	94	59	37	18	10
	±16	12	8	8	9	7	6	5

but at higher temperatures this relation is less apparent. A quadratic or even a cubic equation could be justified as more exactly describing the effect of age on a particular sample of milk. Uncertainty as to an exact definition of zero age, and as to such variables as were suggested in the introduction make the more limited application of such equations more important than their greater precision.

There is a wide field for speculation as to possible physical causes for this relationship. Recovery from loss of viscosity on pasteurization may be the principal cause. Changes in ion concentration due, e.g., to heating, to natural enzymes, or to bacteria may have some importance. This study is not intended to replace the natural assumption that the best way to know a viscosity under given conditions is to measure it under those conditions. However, if numerous viscosity determinations are desired simultaneously, it is impractical to measure them all at once. It is for such a situation that the equation $\eta = a + b \log \text{age}$ was devised.

The average slopes obtained by determining viscosity at specific temperatures, irrespective of homogenization pressures, are shown at the bottom of Table 1. For convenience in estimating viscosity at various ages from a known viscosity at a specific age for milks similar in composition and pretreatment to that described in this manuscript, a conversion chart is presented in Figure 3. For milks of different composition or pretreatment, similar charts can be constructed as needed. Age 10 hours and zero change in viscosity were taken as the

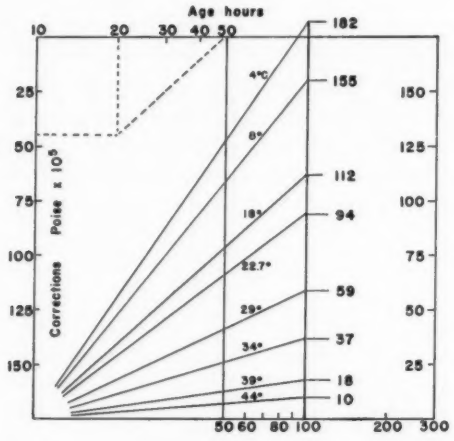

FIG. 3. Corrections for converting viscosities of pasteurized milk from one age to another, at various temperatures. Dotted lines illustrate correction from a measured value at age 20 hours to a desired value at age 50 hours.

origin at the lower left corner of a sheet of semi-log paper. Ages were abscissas on the log scale, and changes in viscosity were ordinates on the uniform scale at right of chart. Each regression line passed through the origin. The other point used to determine the slope at a given temperature was at age 100 hours and was the ordinate equal to the average slope calculated for this temperature in Table 1.

To adjust a viscosity from a measured age to a younger desired age, a line was drawn through the desired age on the lower age scale and parallel to the slope at the temperature of measurement. The ordinate of this line at the age when viscosity was measured was the correction subtracted from the measured viscosity. To convert a measured viscosity to an older age, the desired age on the upper scale was used and the correction was measured downward from the upper left corner of the chart. This correction was added to the measured viscosity.

An illustration will serve to demonstrate the use of the chart. Assume that a sample of milk had a measured viscosity of 3.400 cps. measured at 18° C. after aging 20 hours at 4° C. What would be its viscosity after aging for 50 hours? If a line parallel to the line marked 18° C. passes through the upper age scale at 50 hours, it cuts the vertical line at age 20 hours, 45 correction units below the upper age scale. The viscosity at 50 hours would be 3,400 + 45 = 3,445 poises × 10^5, or 3.445 cps.

If many adjustments are to be made and more precise corrections are desired, it is convenient to redraw the chart on a piece of semi-log graph paper and to mount the sheet with its uniform lines perpendicular to the working edge of a drawing board. A protractor can then be set parallel to the slope and moved to any desired position.

SUMMARY

A slightly modified Ostwald viscometer is described with which small but definite differences in the viscosity of milk were measured. The increases in viscosity of both nonhomogenized and homogenized pasteurized milk measured at temperatures ranging from 4° to 44° C. as a result of aging or storage of the milk at 4° C. were found to be approximately a linear function of the logarithm of the age.

A chart is presented by which the viscosity of milk containing 4.0 ± 0.05% fat and 13.0 ± 0.10% total solids and pasteurized at 62° C. for 30 minutes can be estimated from a known viscosity value at any known age within the age range of 0 to 300 hours. Similar charts can be prepared for milk of different composition and pretreatment.

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A PLAN FOR THE RAPID DETERMINATION OF THE MAJOR CATIONS IN MILK

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In studying the effects of the major cations present in milk upon its properties, a routine analytical procedure that is rapid and accurate is needed. The time-consuming steps involved in eliminating interfering ions in the usual chemical methods for sodium *(8)* and potassium *(10)* can be avoided by flame photometry *(1, 2, 3, 5)*. Keirs and Speck *(7)* and Krienke and Gammon *(9)* have adapted this technique for the determination of calcium, sodium, and potassium in milk but were unable to accurately measure the magnesium content because of its low concentration and the low intensity of its excited wave length. However, magnesium and calcium can be determined rapidly with ethylenediamine tetraacetate as the titrating reagent and suitable indicators by the method of Schwarzenbach *et al. (13)*. Both this method and the flame-photometric determination of calcium require ashing or wet digestion to remove the interfering organic matter and subsequent treatment to remove the phosphates and other interfering ions. However, Jenness *(6)* in applying the method of Schwarzenbach to milk found that a milk serum prepared by acid precipitation followed by treatment with an anion-exchange resin in the acetate form could be analyzed directly for calcium and magnesium and was superior to the other methods of sample preparation, since it requires less time than ashing and less exchange resin than wet digestion.

Trials of Jenness' procedure for the determination of calcium and magnesium in the milk serum prepared by acid precipitation yielded satisfactory results for the total calcium and magnesium analyses, but some difficulty was encountered in identification of the color at the end point in the calcium titrations. Therefore, milk serums were prepared according to Jenness' method modified so as to eliminate sodium ions from the reagents, and the possibility of determining sodium, potassium, and calcium directly by flame photometry was explored. The investigation resulted in a plan for the determination of the major cations in milk requiring considerably less time than other available procedures.

METHOD

Preparation of the milk serum. Ten ml. of the milk sample is diluted with 20 ml. of deionized water in a 100-ml. beaker. Two ml. of 1 *N* HCl is added with stirring, and the sample is allowed to stand for 10 minutes. The sample then is adjusted to a pH of 4.0-4.1 with 0.5 *N* NH_4OH, quantitatively transferred to a 100-ml. volumetric flask, and made up to volume with deionized water. After thorough mixing, the sample is filtered through a Whatman No. 42 filter paper.

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Thirty ml. of the clear filtrate is shaken with 15 g. of anion-exchange resin (Nalcite SAR) in the chloride form in a 125-ml. Erlenmeyer flask on a mechanical shaker (Burrell Wrist-Action Shaker, model BB) for 15 minutes. The resin was previously prepared by rinsing it three times with two and one-half times its weight of deionized water to remove any NaCl or other undesirable contamination present and dried by blotting between filter papers. The solution is then filtered through a Whatman No. 42 paper into a 100-ml. volumetric flask. The resin is washed three times with 20 ml. of deionized water, and the washings and the filtrate are made up to volume with deionized water.

Composition of standard solution. Stock solutions (0.1 *N*) of sodium and potassium are prepared from the reagent-grade NaCl and KCl. A stock solution of calcium (0.1 *N*) is made by dissolving reagent-grade $CaCO_3$ in a minimum amount of concentrated HCl, C.P., and diluting to volume with deionized water. From these stock solutions various working-standard solutions are prepared. Reagent-grade lactose, NH_4Cl, and DL-*a*-alanine are included in all the standard solutions in order to simulate the milk serum sample. The DL-*a*-alanine is added with the expectation that it will contribute to the flame background in the photometer in a manner similar to the milk proteins remaining in the serum, since its elementary composition very nearly approximates that of the common milk proteins. The amount of DL-*a*-alanine to be added was calculated from the results of nitrogen analysis of a milk serum prepared with NaOH instead of NH_4OH. Lithium is added as LiCl for the internal standard. The compositions of the various working-standard solutions are given in Table 1. These standards have been found to be stable for 3 to 4 weeks when stored at ~5° C.

Calibration curves. With these working-standard solutions, the calibration curves for sodium, potassium, and calcium are determined with a Perkin-Elmer Flame Photometer, model 52-C, according to the internal-standard procedure described in the manual *(12)* with an acetylene flame at a pressure of ~25 mm. of Hg. Figure 1 illustrates typical curves.

Correction for the interference of sodium in the calcium determination. Preliminary studies indicated that the only interference encountered in these procedures was that of sodium in the calcium determination at 5540 Å. Therefore, the effect of different amounts of sodium upon the calcium determination must be evaluated. Sodium working-standard solutions are prepared as described above except that all solutions contain 5 p.p.m. of lithium. The apparent calcium contents in these solutions are determined at the wave length of calcium (5540 Å). After the zero and gain adjustment have been made in the same manner as in establishing the calibration curves, a standard sodium solution is atomized, and the internal-standard dial reading is determined. By referring to the calibration curve for calcium, two calcium standard solutions are selected which yield internal-standard settings so that one is slightly lower and the other slightly higher than the standard sodium solution. Finally, the internal-standard dial reading for the standard sodium solution is confirmed. From the four readings obtained, the apparent calcium content in the sodium solution is calculated according to one of the formulas in Table 2, depending upon which standard solu-

TABLE 1
Composition of the standard solutions for flame photometry

Sodium standards							
NaCl	KCl	$CaCl_2$	$MgCl_2$	NH_4Cl	Lactose	DL-α-Alanine	LiCl
(meq/l)							*(p.p.m. Li^+)*
0.40	0.20	0.35	0.05	7.65	0.83	9.30	20
0.30	0.20	0.35	0.05	7.65	0.83	9.30	20
0.20	0.20	0.35	0.05	7.65	0.83	9.30	20
0.10	0.20	0.35	0.05	7.65	0.83	9.30	20
0.00	0.20	0.35	0.05	7.65	0.83	9.30	20
Potassium standards							
KCl	NaCl	$CaCl_2$	$MgCl_2$	NH_4Cl	Lactose	DL-α-Alanine	LiCl
(meq/l)							*(p.p.m. Li^+)*
0.40	0.10	0.35	0.05	7.65	0.83	9.30	20
0.30	0.10	0.35	0.05	7.65	0.83	9.30	20
0.20	0.10	0.35	0.05	7.65	0.83	9.30	20
0.10	0.10	0.35	0.05	7.65	0.83	9.30	20
0.00	0.10	0.35	0.05	7.65	0.83	9.30	20
Calcium standards							
$CaCl_2$	NaCl	KCl	$MgCl_2$	NH_4Cl	Lactose	DL-α-Alanine	LiCl
(meq/l)							*(p.p.m. Li^+)*
0.80	0.10	0.20	0.05	7.65	0.83	9.30	5
0.60	0.10	0.20	0.05	7.65	0.83	9.30	5
0.40	0.10	0.20	0.05	7.65	0.83	9.30	5
0.20	0.10	0.20	0.05	7.65	0.83	9.30	5
0.00	0.10	0.20	0.05	7.65	0.83	9.30	5

tion yields a reading closer to that of the unknown. The apparent calcium contents of the remaining sodium solutions are determined in a similar manner.

In order to evaluate the correction necessary for the error introduced by the presence of sodium, the true calcium concentration (0.35 meq/l) in the standard sodium solution is subtracted from the apparent calcium concentration. These corrections are reported in Figure 2 as a function of the sodium concentration. Therefore, for an observed concentration of sodium in the milk serum, the proper correction for the observed calcium content can be determined from this graph.

Determination of sodium, potassium, and calcium in milk serum. Ten ml. of the resin-treated serum is transferred to each of two 50-ml. volumetric flasks. For the sodium and potassium determination, 1 ml. of a LiCl solution (1,000 p.p.m. Li^+) is added to the contents of one of the flasks. For the calcium determination, 0.25 ml. of the same LiCl solution is added to the contents of the other flask. After diluting to volume with deionized water, these solutions are analyzed for sodium, potassium, and calcium at the appropriate wave lengths. For each element the zero and gain adjustments are made as before, and the internal-standard dial reading is determined for the unknown. From the appropriate calibration curve, two standard solutions are selected which yield internal-standard dial settings so that one is slightly lower and the other is slightly higher than the unknown. The internal-standard dial reading for the unknown is again confirmed and the concentration in the unknown is then calculated ac-

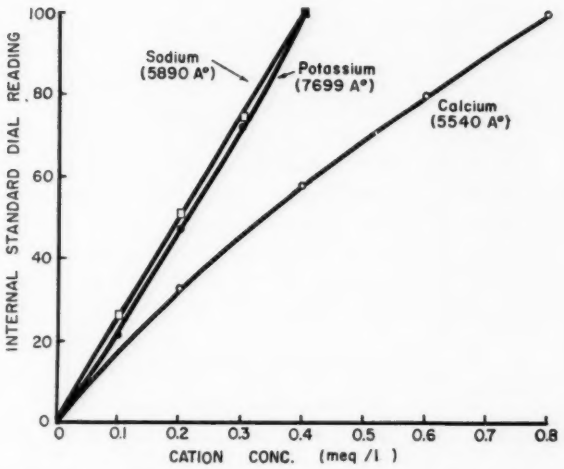

FIG. 1. Typical calibration curves for the flame photometer.

cording to Table 2. The observed calcium concentration must be corrected for sodium interference as described previously.

Determination of magnesium. Since the concentration of magnesium in milk cannot be determined accurately by flame photometry, the total calcium and magnesium content of the resin-treated serum is first determined according to the method described by Jenness *(6)*. The magnesium content is then obtained by subtracting the calcium concentration as obtained by the flame photometer from the total calcium and magnesium value.

RESULTS

The effect of different amounts of anion-exchange resin upon the completeness of phosphate removal. In order to determine the calcium and magnesium content

TABLE 2

Calculation of unknown concentration from flame photometer data

Solution	Concentration	Internal standard dial reading
Unknown	C	X_1 (first reading) X_2 (second reading)
Higher standard	S_1	Y_1
Lower standard	S_2	Y_2
Formulas:		
X nearer to Y_1:	$C = S_1 - \dfrac{(S_1 - S_2)\left[Y_1 - \dfrac{(X_1 + X_2)}{2} \right]}{Y_1 - Y_2}$	
X nearer to Y_2:	$C = \dfrac{(S_1 - S_2)\left[\dfrac{(X_1 + X_2)}{2} - Y_2 \right]}{Y_1 - Y_2} + S_2$	

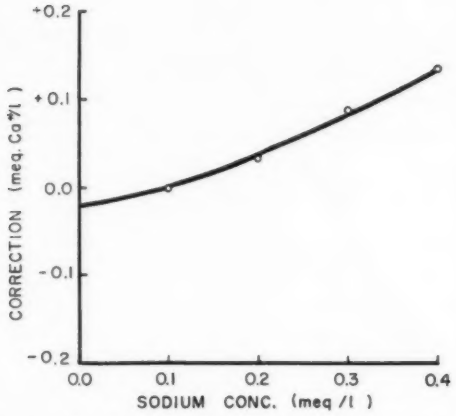

FIG. 2. The interference of sodium on the determination of calcium at 5540Å with the flame photometer.

in the various samples, it was necessary to remove the phosphates and similar interfering ions. Jenness accomplished this by passing an aliquot of the milk serum through an anion-resin exchange column (Duolite A-4). Since the batch process with a different resin was employed in this study, it was necessary to determine the amount of anion-exchange resin required per batch for the complete removal of inorganic phosphorus. Therefore, 30 ml. of a clear milk filtrate prepared in the same manner as described in the method was pipetted into each of six 125-ml. Erlenmeyer flasks and 2.5, 5.0, 7.5, 10.0, 15.0, and 20.0 g. of the washed resin were added, respectively. After the flasks were shaken for 1 hour, the samples were treated in the manner indicated under the preparation of milk serum, and the inorganic phosphorus content of the various samples was determined according to the method described by Fiske and Subbarow *(4)*. The results are given in Figure 3. It can be seen that 15 g. of the resin per 30 ml.

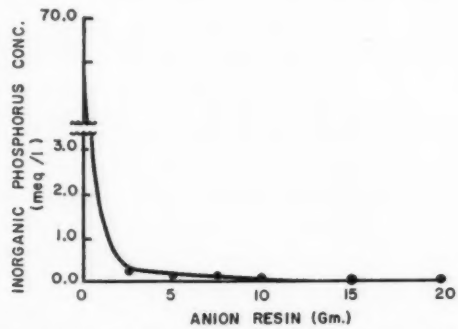

FIG. 3. The effect of the amount of anion-exchange resin (Nalcite SAR) upon the inorganic phosphorus content of the milk serum.

of milk serum is more than sufficient to remove completely the inorganic phosphorus within the error of the method of analysis and therefore should provide an adequate safety factor for variations in the milk samples.

Effect of time of resin treatment upon the completeness of removal of inorganic phosphorus from the milk serum. In order to determine the time necessary for resin treatment of the milk serum, four 30-ml. samples of the clear milk filtrate were mixed with 15 g. of anion-exchange resin and shaken on the mechanical shaker for 15, 30, 45, and 60 minutes, respectively. The samples were treated as described before, and their inorganic phosphorus contents were determined. The results are presented in Figure 4. The data indicate that shaking the samples for 15 minutes is sufficient for the complete removal of inorganic phosphorus within the error of the method.

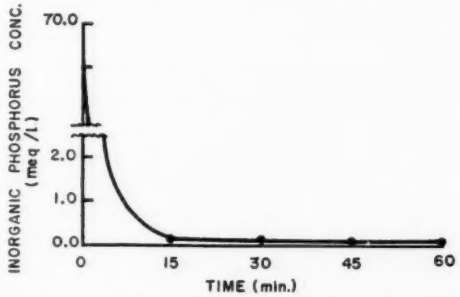

FIG. 4. The effect of the time of resin treatment upon the removal of inorganic phosphorus from the milk serum.

A comparison of the method with the ashing technique. In order to determine whether the results obtained with the serum samples prepared in the manner indicated are sufficiently reproducible and comparable to those obtained from an analysis of milk ash solution, the sodium, potassium, calcium, and magnesium content of ten different milk samples (five whole milk and five skimmilk) were determined in duplicate by both the serum method and the ashing procedure. Since the preliminary studies indicated that ashing of the milk samples in porcelain crucibles gave low potassium values and high sodium values, platinum crucibles were employed for ashing the milk samples in the present study. In the ashing procedure, 10 ml. of the milk sample was placed in a platinum crucible and 0.5 ml. of glacial acetic acid was added to coagulate the proteins. The sample was dried in an electric oven at 110° C. overnight and ashed in a muffle furnace at 550° C. for 24 hours. The ash was moistened with a little deionized water and dissolved with 2 ml. of 1 *N* HCl by warming the solution and allowing it to stand for 30 minutes. The solution was quantitatively transferred to a 100-ml. volumetric flask and made up to volume with deionized water.

For analysis of the ash solution with the flame photometer, two 10-ml. portions of the ash solution were diluted approximately to 50 ml. each with 0.01 *N*

HCl in 100-ml. beakers, and excess lead chloride (0.5-1.0 g.) was added to precipitate the phosphates and other interfering ions according to the method of Krienke and Gammon *(9)*. The solutions were heated to boiling and maintained at that temperature for 15 minutes, cooled to 15°-20° C., and decanted into 100-ml. volumetric flasks. The precipitates were washed three times with 10 ml. of 0.01 *N* HCl and the washings were transferred to the flasks. Two ml. of LiCl solution (1,000 p.p.m. Li^+) was added to the solution employed for sodium and potassium determination, and 0.5 ml. was added for the calcium determination. The solutions were made to volume with 0.01 *N* HCl, stored at 36°-38° F. overnight, and filtered in the cold to remove the insoluble $PbCl_2$. Then the samples were warmed to room temperature and analyzed for sodium, potassium, and calcium with the flame photometer. Since the composition of the sample prepared in this manner was different from that of the milk-serum samples, appropriate standard solutions were prepared to include $PbCl_2$, and calibration curves were established as described before.

For the determination of magnesium, 30 ml. of the ash solution was treated with 15 g. of anion-exchange resin and analyzed in the same manner as the milk serum. The results are reported in Table 3.

Statistical analysis of the data indicate that the method used for the preparation of the sample for the flame photometer, either milk serum or ash solution, does not sufficiently influence the observed sodium, potassium, calcium, and magnesium content of the milk samples to exceed the accuracy required in most analytical work. In fact, in all cases but the interaction terms between milk and method for calcium and potassium, there are no significant effects observed. As would be expected, there is a significant difference in the major cation concentration between whole milk and skimmilk because of the presence of fat in the whole milk, and also between the different milk samples. The results of the potassium analyses indicate a significant interaction between the milk analyzed and the method of preparation of the sample at the 1% level. This suggests that the method of preparation does influence the results of analysis, but the direction of the influence is a function of the milk to be analyzed. The mean-square variance for this interaction suggests that the expected variance to be encountered between methods of preparation of the sample for single analysis will be 0.074 or a standard deviation of 0.272 meq/l, less than 1% of the measured values. Such a variation does not exceed the required accuracy for this study. The same conclusion can be reached with regard to calcium where the interaction variance is significant only at the 5% level.

Application of the scheme to the analysis of major cations in a milk dialysate. To determine whether treatment of milk dialysates with anion-exchange resin without ashing would make possible their direct analysis according to the proposed plan, a milk dialysate was prepared by dialyzing each of three 60-ml. portions of deionized water against 20-l. portions of pasteurized skimmilk at 36°-38° F. for 120 hours. An aliquot of the combined dialysates was treated with resin as described under the method and analyzed for sodium, potassium, calcium, and magnesium. For comparison, a portion of the dialysate was ashed

TABLE 3

A comparison of the ash and serum methods of sample preparation for the sodium, potassium, calcium, and magnesium analyses of milk

Milk	Calcium (Flame photometer) (Av. of duplicates)		Magnesium (by difference) (Av. of duplicates)		Sodium (Flame photometer) (Av. of duplicates)		Potassium (Flame photometer) (Av. of duplicates)	
	Ash	Serum	Ash	Serum	Ash	Serum	Ash	Serum
	(meq/l)							
Whole milk	58.47	58.80	7.85	7.61	17.52	17.44	35.09	34.87
	54.93	54.78	11.21	10.95	17.37	17.39	36.09	35.99
	54.13	54.16	9.58	9.54	17.12	17.14	37.18	37.55
	53.30	53.30	9.33	9.19	18.31	18.48	37.68	37.91
	54.45	54.15	9.25	9.55	16.74	16.88	37.23	37.10
Skim-milk	59.94	60.47	7.94	7.95	17.38	17.23	40.58	40.56
	59.92	59.56	10.53	10.62	19.28	19.46	36.26	36.37
	61.78	61.49	6.37	6.40	18.32	18.45	38.57	38.03
	60.46	60.58	9.31	9.21	17.92	18.18	39.58	40.14
	57.80	57.47	9.41	9.88	18.20	18.30	38.56	38.96

Analysis of variance:

Source of variance		Degrees of freedom	Mean square	Significance
Sodium				
Method	(m)	1	0.0630	n.s.
Skimmilk vs. whole milk	(T)	1	6.9720	0.1%
Individual milk	(M)	8	1.8067	0.1%
m × T		1	0.0076	n.s.
m × M		8	0.0176	n.s.
Error		20	0.0152	
Potassium				
Method	(m)	1	0.0790	n.s.
Skimmilk vs. whole milk	(T)	1	42.8080	0.1%
Individual milk	(M)	8	7.7315	0.1%
m × T		1	0.0414	n.s.
m × M		8	0.1226	1%
Error		20	0.0244	
Calcium				
Method	(m)	1	0.0000	n.s.
Skimmilk vs. whole milk	(T)	1	236.1000	0.1%
Individual milk	(M)	8	13.0450	0.1%
m × T		1	0.0083	n.s.
m × M		8	0.0889	5%
Error		20	0.0363	
Magnesium				
Method	(m)	1	0.0016	n.s.
Skimmilk vs. whole milk	(T)	1	4.1409	0.1%
Individual milk	(M)	8	8.1571	0.1%
m × T		1	0.0749	n.s.
m × M		8	0.0746	n.s.
Error		20	0.0319	

as described before, and the ash solution was analyzed for the major cations. Because of the difference in composition between the dialysate and the milk, it was necessary to prepare appropriate working-standard solutions and calibration curves for both the resin-treated and the ashed dialysate in a manner similar to that described in the method. The results of this experiment with their statistical analysis are reported in Table 4.

TABLE 4

A comparison of the ash and resin treatment methods of sample preparation for the sodium, potassium, calcium, and magnesium analyses of milk dialysates

Cations		Ash solution (Av. of duplicates)	Dialysate (Av. of duplicates)
Total calcium and magnesium	*(meq/l)*	22.32	22.11
Calcium	*(meq/l)*	18.75	18.50
Magnesium	*(meq/l)*	3.52	3.60
Sodium	*(meq/l)*	17.99	17.87
Potassium	*(meq/l)*	39.95	39.63

Analysis of variance:

Source of variance	Degree of freedom	Mean square	Significance
	Sodium		
Method	1	0.0144	n.s.
Error	2	0.0037	
	Potassium		
Method	1	0.1056	5%
Error	2	0.0042	
	Calcium		
Method	1	0.0650	n.s.
Error	2	0.0233	
	Magnesium		
Method	1	0.0090	n.s.
Error	2	0.0169	

The observation that the resin-treated dialysate yields a potassium content significantly different at the 5% level from that of the ashed dialysate is consistent with the observed significance of the interaction term in the milk analyses. Its variance is of the same order of magnitude, and therefore it may be assumed that variances due to the method of preparation will not sufficiently influence the observed potassium content to exceed the accuracy required in most analytical work. Therefore, it may be concluded that this plan of analysis may be successfully employed for the determination of the major cations in the dialysate without ashing.

SUMMARY

A plan for the rapid determination of the major cations in milk has been described. With this procedure, the standard deviations of the analyses for various ions are as follows: sodium, 0.124 meq/l or 0.70%; potassium, 0.157 meq/l or 0.42%; calcium, 0.191 meq/l or 0.33%; and magnesium, 0.179 meq/l or 1.97%. These values indicate sufficient precision for most purposes. The agreement between the results of analysis with this scheme and those with the usual procedure is satisfactory.

Resin treatment can satisfactorily be employed as a preparatory treatment for cation analysis of a milk dialysate.

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RADIATION PRESERVATION OF MILK AND MILK PRODUCTS
I. BACKGROUND AND PROBLEMS[1]

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The past decade has witnessed a great many advances in food research. One of these, which has attracted a tremendous amount of attention, is radiation sterilization or "cold sterilization" of foods, drugs, and pharmaceuticals.

From a modest research effort of relatively few thousands of dollars in 1945, concentrated in three laboratories in this country, radiation sterilization research has grown tremendously until today there are some 50 or more organizations in this country actively engaged in one or more facets of research in this exciting new field of sterilization *(1-6)*. The total dollar volume is difficult to assess completely; however, it is believed that some three million dollars per year are being spent in research in this field in the attempt to achieve the ultimate goal of complete sterilization without heat and without any adverse effects.

The phenomenon of destruction of microorganisms by bombardment with ionizing energy of some type has been known for years, in fact, almost as early as the discovery of X-rays and the radioactive elements. However, it was not until World War II and the subsequent development of tremendous sources of ionizing energy as part of the Manhattan Project that research work in this field became active. The cooperation and sponsorship of research by the Atomic Energy Commission in 1951 and more recently by the Department of the Army has resulted in large measure in the participation of numerous investigators, each well skilled in his own particular discipline.

This has had an important role in increasing the research effort in this area. However, industry is itself actively engaged in radiation research and is sponsoring a relatively large portion of university research.

UNITS AND TERMINOLOGY

In order to better understand work in this field, it is necessary to realize the energy ranges necesary, as well as to acquire some knowledge of the units of radiation and the terminology used in the succeeding papers in this series that are peculiar to this particular field.

Roentgen (r): quantity of X- or γ-radiation which, under standard conditions of temperature and pressure (0° C. and 760 mm. Hg), produces one electrostatic unit of charge (e.s.u.) of either sign.

Roentgen-equivalent(s)-physical (rep): quantity of ionizing energy, other than X-rays or γ-rays, which produces, per gram of tissue, an ioni-

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zation equivalent to the quantity of ionization produced in air by 1 roentgen of γ or X-rays (1 r or 1 rep $\simeq$ absorption of 93 ergs per gram of tissue).

Erg: the unit of work or energy in the C-G-S system
1 erg = 1 cm-dyne
1 erg = 10^{-7} joules

Joule: a unit of energy or work
1 joule = 1 watt-second

Watt (w.): the practical unit of power
1 watt = 10^7 ergs/second or 1 joule/second

Electron volts (e.v.): the basic energy unit in nuclear physics. 1 e.v. is the kinetic energy acquired by an electron in falling freely through a potential drop of one volt.
1 e.v. = 1.6020×10^{-12} ergs.
Since the electron volt represents such a minute amount of energy, it is customary to calculate in units of million electron volts (m.e.v.), where 10^6 e.v. = 1 m.e.v.

Electrons (e, e^-, β^-): a fundamental particle, the smallest known particle having a negative electric charge and found in the extranuclear shells of atoms.

Cathode rays: streams of electrons emitted from the cathode of an evacuated tube.

Beta rays or beta particles (β^-): negatively charged particles, i.e., electrons ejected from the nuclei of radioactive elements during the radioactive decay process.

X-rays: Penetrating electromagnetic waves of relatively short wave length which are produced by allowing a stream of cathode rays to infringe on a metal target within an evacuated tube.

Gamma rays: electromagnetic radiation of nuclear origin, of short wave length, emitted by the nuclei of certain atoms during their radioactive decay. They have identical properties as X-rays of equivalent energy level.

ENERGY REQUIREMENTS FOR THE DESTRUCTION OF VARIOUS BIOLOGICAL SPECIES

The energy requirements for the destruction of various biological species differ. Man is the most susceptible; the data in Table 1 show the relatively low energy requirements which affect man as compared with lower forms of life. This clearly illustrates the need for proper personnel protection when working in this field. Microorganisms and other lower forms of life are relatively quite resistant and require much larger amounts of energy for their destruction; this illustrates the need of powerful sources of radiation.

TABLE 1
Lethal doses for the destruction of various biological species

Species	Approximate lethal dose
	(rep)
Man	600-860
Insects	25,000-100,000
Nonsporulating bacteria	100,000-500,000
Yeasts	100,000-500,000
Molds	100,000-500,000
Bacterial spores	1,500,000-4,000,000

HOW RADIATIONS DESTROY MICROORGANISMS

One of the fundamental properties of ionizing energy (as the name suggests) is its ability to cause ionization in the medium it traverses. This ionization of the solvent medium produces free radicals, activated molecules, and ions both oxidative (H_2O^+, OH, H_2O_2, etc.) and reductive (H) in nature. Thus, anything in the medium which is reducible (e.g., methylene blue) can be reduced and any solute which is oxidizable in nature (e.g., vitamin C) will be oxidized.

A number of workers have theorized that the destruction of microorganisms may be effected by the passage of an ionizing particle or quantum of energy through, or in close proximity to, a sensitive portion of the cell. This causes direct "hit" on this target, ionization in this sensitive region, and the subsequent demise of the organism.

Although in the main, since microorganisms on a molecular scale are relatively large, they are destroyed by direct hits, some destruction due to indirect means (ionization of the solvent) takes place. The extent of the destruction due to indirect action is based in part on the particular bacterial species and on other conditions to be discussed later.

UNDESIRABLE SIDE REACTIONS

On a molecular scale, amino acids, vitamins, and other nutrients in foods are infinitely smaller than microbes, the volume occupied by bacteria in the food material is greater, and the probability of the vitamins, etc. being affected by direct hits therefore is very small.

On the other hand, they are very susceptible to the ionization products and thus are indirectly affected by the radiation process. The nature of the effect is dependent on the chemical structure of the particular nutrient in question, whereas the extent of the effect (for a given dose of radiation) is dependent on several conditions:

1) Physical state of medium
   a. Moisture content
   b. Temperature
2) Relative sensitivity of the particular solute molecule
3) Concentration of the solute molecule
4) Presence of other solutes

Although it is undoubtedly beyond the scope of the present paper to go into these in any detail (see references cited), it is desirable to point out that free

radical formation is decreased by lowering the temperature or by having less solvent (moisture) present. The more solutes that are present to compete for the free radicals, the less the effect on any one solute. Thus, although a given nutrient (e.g. a vitamin) is relatively easily affected in the pure state, when a number of other nutrients are present, e.g., in foods, quantitatively the effect on any single nutrient is much less because of this competition for the free radicals.

Although the number of molecules changed in any given nutrient is the same (for the same dose) regardless of concentration, percentagewise the effect is less, the greater the concentration (Figure 1). This general statement does not hold in cases of very dilute or extremely concentrated solutions.

Color and flavor molecules may be affected by irradiation bombardment in the same way that nutrients are affected. These indirect effects of radiations are responsible for the undesirable side effects of radiation.

Attempts to date to minimize these side effects have been predicated on lowering the numbers of free radicals by means of:

1) Reducing the temperature (irradiation in the frozen state)
2) Lowering the oxygen content
3) Reducing the moisture content

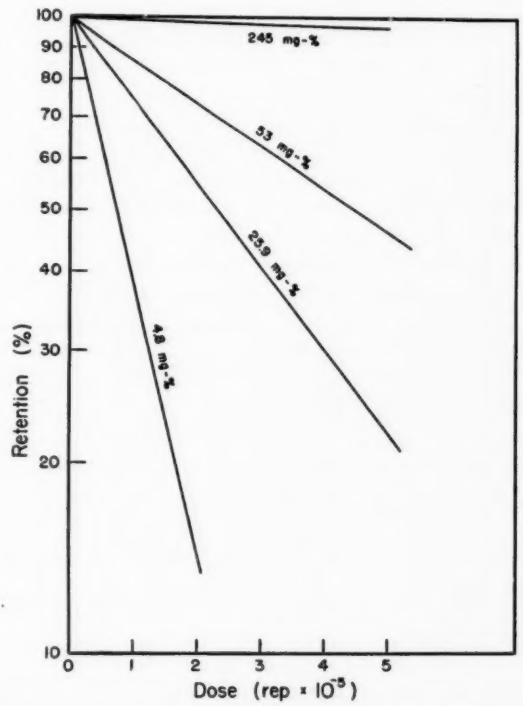

FIG. 1. The effect of cathode rays on aqueous solutions of vitamin C of different concentrations.

A fourth means has been devised, which is based on this competition for free radicals, namely, the addition of compounds that more readily react with free radicals than the solute molecules.

None of the above means, however, has been found to be completely effective in preventing the undesirable side effects in all foods.

To date, milk and other dairy products have been found to be most sensitive to ionizing energy, and work was undertaken in these laboratories to study the basic effects on milk and dairy products in an attempt to discover means whereby an acceptable product might be produced.

Analysis of the fundamental direct and indirect effects of radiation indicates that the most desirable situation to achieve is one wherein the indirect effect is minimized to the greatest possible degree and the direct effect utilized solely for destruction of microbes. Even in this optimal situation, the same solute molecules will be affected by direct hits on them; however, the probability of these occurring is very small indeed.

Fluid milk has been shown to be very sensitive to radiations even under the best technological conditions as described above. Radiation-induced off-flavor threshold detection levels occur at doses approximately 1/100th of the levels required for sterilization. This illustrates the enormity of the problem.

To produce an acceptable radiation-treated product it is necessary to reduce the indirect effect to almost nil; thus a drastic reduction of the quantity of free radicals produced must be achieved or it may even be necessary to ascertain some means of preventing free radical production entirely.

The present series of five papers describes the results of research on dairy products conducted over the past year in these laboratories. The last paper in this series presents a new processing device for the laboratory production of radiation-sterilized milk with no undesirable side reactions.

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RADIATION PRESERVATION OF MILK AND MILK PRODUCTS. II. OFF-FLAVORS IN MILK AND CREAM INDUCED BY IONIZING RADIATIONS AS JUDGED BY ORGANOLEPTIC TESTS[1,2]

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The effect of ionizing radiations on the flavor of milk has been qualitatively determined *(8, 12, 21)*. Although considerable research has been conducted on the prevention of undesirable flavor changes in irradiated milk *(7, 8, 15, 23)*, no observations have been reported on the dose levels at which the off-flavors are first perceptible, i.e., the off-flavor detection threshold.

The research described in this paper was undertaken to expand the basic knowledge relating to the comparison of the intensities of radiation-induced off-flavors in cream and various types of whole and skimmilk. The effects of certain pre-irradiation processing treatments and the relation of some of the components of these basic foodstuffs (such as moisture and fat contents) to the development of radiation-induced off-flavor were also investigated. Milk in various forms was selected for this study because of its importance as a food and because it is a complex biological fluid possessing known sensitivity to ionizing radiations.

MATERIALS AND METHODS

The fluid milk and cream used in this study was certified milk and cream *(1)* obtained from a mixed herd of Guernsey and Holstein cows. Pasteurization was accomplished with commercial equipment, by the high-temperature short-time method. Raw skimmilk was prepared by preheating raw whole milk to 40° C. in a 60° C. water bath, separating through a cream separator, and immediately refrigerating in quart bottles at 5° C. Homogenized milk was prepared by preheating pasteurized whole milk to 50° C. and homogenizing at 2,500 p.s.i., followed by immediate refrigeration at 5° C. Milk containing 1% butterfat was obtained by mixing pasteurized whole milk with pasteurized skimmilk in the proportion of 1:3. The dry whole milk and dry skimmilk were commercially produced by spray drying. The evaporated milk also was commercially produced.

The source of ionizing radiations was a Van de Graaff electrostatic generator rated at three million volts (m.e.v.) constant potential *(11, 25, 26, 28)*. Samples were conveyed under the electron beam on a constant-speed belt and were contained in standardized 80-mm. glass crystallizing dishes covered with aluminum

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[2] This material is taken in part from a thesis by the senior author, to be submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Food Technology at the Massachusetts Institute of Technology.

foil 0.0008 in. thick. The quantity of sample contained in each dish was dependent on the density (specific gravity) of the sample and the operating voltage of the generator. Effective electron penetration was estimated to be 0.9 g. per square centimeter at 2.7 m.e.v. and 1.0 g. at 3.0 m.e.v. *(27)*. Doses delivered were attained by preselection and maintenance of proper beam current and operating voltage.

Fresh fluid milk and cream samples were obtained from the milk plant 12 to 24 hours prior to treatment and were stored at 5° ± 3° C. until used. Powdered skimmilk and powdered whole milk were reconstituted by mixing 105 g. and 157 g., respectively, with 1 l. of distilled water. The powders were reconstituted about 12 hours prior to irradiation and were stored at refrigerator temperature until irradiated. Skimmilk and whole milk powders irradiated in the dry form prior to reconstitution were chilled by refrigerated storage before irradiation and reconstituted in cold distilled water immediately after irradiation.

Irradiated fluid samples were collected in 4-l. flasks and distributed in quart milk bottles for refrigerated storage prior to testing. Irradiated powdered samples were placed in quart bottles containing a predetermined amount of distilled water, sufficient to reconstitute the powder from several irradiation dishes. All samples were irradiated at a temperature between 10° and 16° C.

Milk for chemical analysis was preserved with formaldehyde and stored at 5° C. until tested. Determinations of the total solids of the fluid milk, the evaporated milk, and the heavy cream were made by the hot air oven method *(2)*, and of the dry whole and dry skimmilk powders by the vacuum oven method *(4)*. The fat content in the fluid milk was determined by the Babcock method *(2)*, and in the evaporated milk, heavy cream, and dry whole and skimmilk powders by the Roese-Gottlieb method *(2)*.

Nitrogen determinations were made by the Kjeldahl method *(4)*. Protein was calculated from the nitrogen determinations by multiplying the percentage of nitrogen by 6.38. The determination of ash was made by igniting samples in a muffle furnace at not over 550° C. *(2)*. The percentage of carbohydrate was calculated by difference.

Bacterial counts were made by the agar plate method *(2)*. The incubation temperature was 32° C.

Taste tests were conducted in mid-morning and mid-afternoon, at least 1 hour and not more than 8 hours after irradiation. The taste panel was composed of 18 to 25 judges selected from the departmental staff and students. Both men and women, some smokers and some nonsmokers, participated. All had previous experience in judging flavor quality and in threshold testing. Judges were familiarized with the characteristic radiation-induced off-flavor by being presented, at the first tasting, with samples that had received the greatest irradiation dose.

The test samples were presented as an asymmetric binomial system of the type AA-B, as described by Lockhart *(19)*. This design is more familiarly known as the "triangle test." The samples were served cold, in quantities of 20 to 25 ml. contained in identical, coded, 125-ml. glass beakers. The codes were on tape

attached to the bottom of the beaker to eliminate any code bias *(16)* in the triangle test. Each set of three consisted of either one sample of untreated milk and two samples of irradiated milk or two samples of untreated milk and one sample of irradiated milk.

Combinations of samples were given randomly, and the judges were given no information about the nature of the combinations, except that two of the three samples were similar. Each judge was asked to select the odd sample and, in addition, to specify whether the odd sample was irradiated. Three sets, randomly distributed, were presented to each judge at the same sitting. After the judge had separated and identified the odd sample in each set, he was given the codes of two samples and asked to rate these samples by the hedonic scale method *(22)*. These samples represented both the untreated and the irradiated milk.

The method for calculating threshold dose is explained in some detail, since the application of mathematical line-fitting on log-probability paper to organoleptic testing has not previously been described. According to Guilford *(13)*, a threshold may be taken as the difference in concentration of flavor or odor that can be detected in just 50% of the samples tested. In this paper, the dose of ionizing radiations that produces a flavor difference detectable in 50% of the judgments over and above the correct judgments expected by chance will be defined as the difference threshold dose (DTD). The dose of ionizing radiations that produces a radiation-induced off-flavor recognizable in 50% of the judgments over and above the correct judgments expected by chance will be defined as the recognition threshold dose (RTD).

The percentage of correct judgments above chance was calculated from the equation:

$$Pc = k\,(Po - Pe) \tag{1}$$

as given by Berg *et al. (5)* and previously used by Lockhart and Stanford *(20)*. Pc is the percentage of correct judgments over and above chance, Po is the percentage of correct judgments observed in the test, Pe is the percentage of correct judgments obtained by chance, and k is a constant depending upon the type of binomial design being used.

For the triangle test, $Pe = 33.3\%$, since one-third of the judgments may be correct by chance, and $k = 3/2$. When the judge is also asked to specify whether the odd sample in the triangle test has been irradiated, then Pe becomes 16.7%, since one-sixth of the judgments may be correct by chance, and $k = 6/5$.

Harrison and Elder *(14)* have illustrated how the response obtained as described above can be presented graphically on log-probability paper as a function of stimulus. These authors selected the log-probability coordinates "because flavor perception is expected to vary with the logarithm of the stimulus value (Weber-Fechner Law), and the distribution of scores around the halfway point is assumed to follow the normal probability function." Threshold dose can then be estimated from the dose-response (log-probability) curve at the point where $Pc = 50\%$.

Harrison and Elder designate the ordinate, or Y axis, as the stimulus and the abscissa, or X axis, as the response. Since the response (percentage of correct judgments above chance) is the dependent variable and the dependent variable is customarily designated as Y (contrary to the procedure of Harrison and Elder), the probability coordinate should fall on the Y axis.

To obtain the most precise estimate of the threshold, it is necessary to fit the best straight line mathematically to the response data. This is done by the method of least squares *(3)*. Eisenhart *(9)* points out that when y values are observed for preselected x values and then the observed values of Y are used to estimate X, the best straight line should be fitted to $Y = a + bX$ and then the inverse of this relation, i.e., $X = (Y - a)/b$, should be used to estimate X. Thus, by the method of least squares, $\Sigma(y - \overline{Y})^2$ is minimized.

Logarithm-probability paper transforms both dose and response variables. Such a transformed variable is called a "metameter." The response metameter is the transformation of percentages into the normal-curve deviate. Kelley *(17)* has prepared tables of this transformation. The dose metameter is simply the transformation of dose into log dose.

In calculating the best straight line, $Y = a + bX$, therefore, on log-probability paper by the method of least squares, it is necessary to determine values of a and b objectively when Y (percentage of correct judgments above chance) is transformed to the normal-curve deviate or other response metameter *(10)* and X (dose) is transformed to log dose.

When the normal-curve deviate is used as the response metameter, the best estimated threshold dose can be calculated from the mathematically fitted line by the equation:

$$\text{Threshold dose} = \log^{-1} - a/b \qquad (2)$$

Confidence limits of the threshold dose can be calculated by formulae given by Eisenhart *(9)*, Bliss *(6)*, or Anderson and Bancroft *(3)*.

RESULTS AND DISCUSSION

The results of the triangle taste tests with cream and various types of milk indicate that flavor differences can be detected in these products after they have been irradiated with very low doses of high-voltage cathode rays (Table 1). In addition to the detection of flavor difference, the flavor can also be recognized at these low dose levels as the off-flavor produced by ionizing radiations (Table 2).

With raw whole milk, for example, the recognition threshold dose was estimated from the dose-response curve (Figure 1) to be approximately 13,500 rep. At this dose level, approximately 60% of the microorganisms were inactivated (Table 3). O'Meara *(21)*, working with pasteurized whole milk inoculated with *Escherichia coli* to give a concentration in the milk of 7×10^5 organisms per milliliter, found that at a dose level of 75,000 rep the presumptive test for *E. coli* was negative. In raw whole milk containing 2.75×10^5 organisms per milliliter,

TABLE 1

Flavor difference threshold doses for milk and cream irradiated with high-voltage cathode rays

Dairy product	Difference threshold dose	Confidence limits (95%)		Regression equation[a]
	(rep × 10^{-3})	*(rep × 10^{-3})*		
Fluid whole milk				
Raw	7.39	5.56	9.49	$Y = -4.3272 + 1.1186\ X$
Pasteurized	6.03	2.84	8.85	$Y = -6.0806 + 1.6085\ X$
Past. and homog.	14.29	11.01	17.09	$Y = -7.2159 + 1.7367\ X$
Dry whole milk[b]				
Irr. before rec.	59.68	[c]		$Y = -4.0193 + 0.8416\ X$
Irr. after rec.	20.79	[c]		$Y = -3.3671 + 0.7798\ X$
Fluid skimmilk				
Raw	16.72	9.04	27.42	$Y = -8.5308 + 2.0200\ X$
Pasteurized	17.16	12.65	22.95	$Y = -7.1432 + 1.6869\ X$
Dry skimmilk[b]				
Irr. before rec.	128.20	53.32	501.80	$Y = -6.9969 + 1.3698\ X$
Irr. after rec.	15.34	[c]		$Y = -8.6133 + 2.0518\ X$
Other products				
Pasteurized, 1% butter fat	9.32	[c]		$Y = -5.8606 + 1.4765\ X$
Raw heavy cream	7.52	3.45	11.13	$Y = -3.7788 + 0.9749\ X$
Homog. evap. milk[b]	27.37	[c]		$Y = -4.5290 + 1.0207\ X$

[a] Mathematically fitted best straight line of normal curve deviate *(Y)* corresponding to percentage above chance scores vs. log dose *(X)* on log-probability paper.

[b] Reconstituted milk.

[c] Confidence limits at the 95% level could not be calculated because the slope of the regression line was not significantly different from zero.

sterilization was accomplished at a dose level of 750,000 rep. These pasteurization and sterilization doses serve to give perspective to the severity of the off-flavor problem.

The acceptability of the dairy products studied decreased as the dose and the accompanying off-flavor development increased. Table 4 shows the hedonic scale

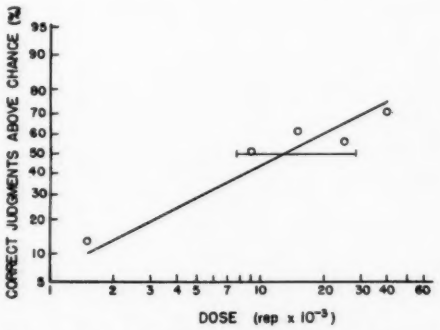

FIG. 1. Relation between dose of high voltage cathode rays on raw whole milk and correct separation and identification of odd sample in triangle test, scored as percentage of judgments above chance.

TABLE 2

Off-flavor recognition threshold doses for milk and cream irradiated with high-voltage cathode rays

Dairy product	Difference threshold dose	Confidence limits (95%)		Regression equation[a]	Proximate analysis				
					Moisture	Fat	Protein	Ash	Carbohydrate
	(rep × 10⁻³)	*(rep × 10⁻³)*			*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Fluid whole milk									
Raw	13.41	7.70	28.29	$Y = -5.0424 + 1.2217\ X$	87.1	4.0	3.0	0.8	5.2
Pasteurized	7.21	4.72	9.46	$Y = -5.7927 + 1.5015\ X$	87.1	4.0	3.0	0.8	5.2
Past. and homog.	17.35	12.59	21.38	$Y = -9.3054 + 2.1950\ X$	87.1	4.0	3.0	0.8	5.2
Dry whole milk[b]									
Irr. before rec.	58.99			$Y = -4.4440 + 0.9315\ X$	2.2	28.2	25.5	5.9	38.2
Irr. after rec.	14.44	9.87	21.36	$Y = -5.6121 + 1.3492\ X$	85.8	4.1	3.7	0.9	5.5
Fluid skimmilk									
Raw	20.23	18.48	23.71	$Y = -11.9662 + 2.7790\ X$	91.2	0.0	3.0	0.8	5.1
Pasteurized	20.06	15.11	27.23	$Y = -8.5688 + 1.9917\ X$	91.2	0.0	3.0	0.8	5.1
Dry skimmilk[b]									
Irr. before rec.	106.70	52.32	199.80	$Y = -4.7563 + 0.9459\ X$	1.6	0.9	34.3	8.3	54.9
Irr. after rec.	14.80	[c]		$Y = -6.7572 + 1.6204\ X$	90.7	0.1	3.3	0.8	5.2
Other products									
Pasteurized, 1% butter fat	13.02	10.00	16.57	$Y = -8.2080 + 1.9949\ X$	90.1	1.0	3.0	0.8	5.1
Raw heavy cream	8.02	0.65	14.84	$Y = -3.5310 + 0.9044\ X$	51.3	44.4	1.4	0.2	2.6
Homog. evap. milk[b]	24.27	[c]		$Y = -4.9981 + 1.1398\ X$	74.3	8.1	6.7	1.6	11.4

[a] Mathematically fitted best straight line of nomal curve deviate *(Y)* corresponding to percentage above chance scores vs. log dose *(X)* on log-probability paper.

[b] Reconstituted milk.

[c] Confidence limits at the 95% level could not be calculated because the slope of the regression line was not significantly different from zero.

TABLE 3

Effect of high voltage cathode rays on the survival of microorganisms in raw whole milk

Dose	Standard plate count[a]/ml	Per cent[b] survival
(rep)		
Control	6,500	
12,500	2,700	41.5 ± 4.5
50,000	1,800	28.8 ± 5.4
100,000	100	1.5 ± 0.4
500,000	0	0
1,000,000	0	0

[a] Bacteria per ml. after incubation at 32° C.
[b] Confidence limits @ 95% probability level.

evaluation of raw whole milk treated with different doses. At a dose level of 20,000 rep a significant flavor difference could be detected at the 1% level, whereas at a level of 10,000 rep, no significant difference could be detected at the 5% level. These results agree with the recognition threshold dose obtained by the triangle test method. Based on the hedonic rating as a scale of acceptability where scores above 5 represent different degrees of "like" and scores below 5 represent different degrees of "dislike," plots have been prepared showing the acceptability of fluid whole milk (Figure 2), fluid skimmilk (Figure 3), powdered milk (Figure 4), and other dairy products (Figure 5). In products on which the mean hedonic rating of the control sample was below 5, low doses of ionizing radiations did not significantly reduce the acceptability further.

Off-flavor threshold dose results obtained by the triangle test method appear to compare closely with the dose at which the taste panel neither liked nor disliked the irradiated samples (mean hedonic rating of 5). This comparison can be made only with the products in which the untreated sample received a mean hedonic rating above 5.

Since at present no instruments other than the human senses can directly describe flavor sensations, the flavor panel was used as a research tool to determine whether flavor induced by ionizing radiations was modified by pre-irradiation treatments or changes in composition of the dairy products. Other objective

TABLE 4

Hedonic scale method evaluation test of irradiated and unirradiated raw whole milk

Dose	Number of judges	Mean score	Standard deviation	Standard error	Significance of differences between scores	Percentage of judges disliking sample
(rep × 10^{-4})						
Control	24	6.00	1.38	0.28	Not significant	16.7
0.5	24	6.09	1.49	0.30	at 5% level	20.8
Control	24	6.59	1.10	0.22	Not significant	4.2
1.0	24	6.25	1.60	0.33	at 5% level	12.5
Control	24	6.21	1.41	0.29	Significant at	8.3
2.0	24	4.71	1.68	0.34	1% level	41.7
Control	24	6.67	0.87	0.18	Significant at	0
4.0	24	3.71	1.72	0.35	1% level	62.5

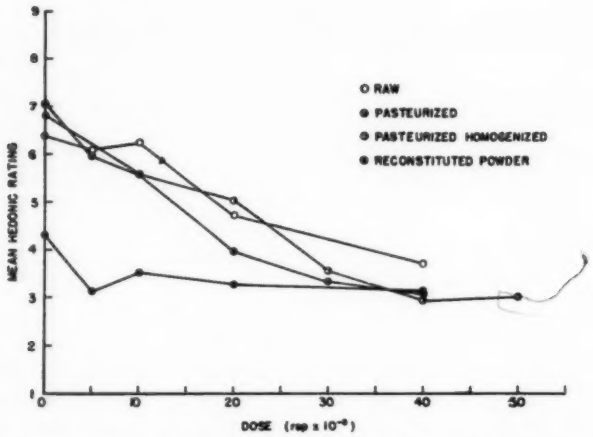

FIG. 2. Mean hedonic ratings of fluid whole milk irradiated with high voltage cathode rays.

analytical flavor techniques have been applied to dairy products and will be reported in future papers.

To establish whether significant flavor differences occurred among samples receiving pre-irradiation treatments, it was necessary to establish confidence limits for the radiation-induced off-flavor recognition threshold doses. Confidence limits of 95% about the off-flavor threshold dose were calculated for the products whose dose response curves had slopes significantly greater than zero at this probability.

In fluid whole milk and fluid skimmilk there was no significant difference in the recognition threshold dose between the raw and the pasteurized products.

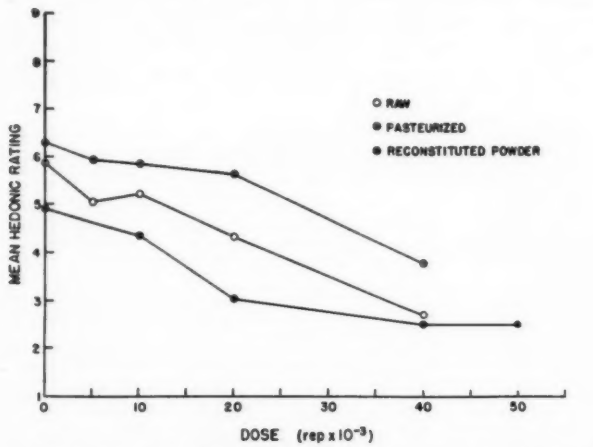

FIG. 3. Mean hedonic ratings of fluid skimmilk irradiated with high voltage cathode rays.

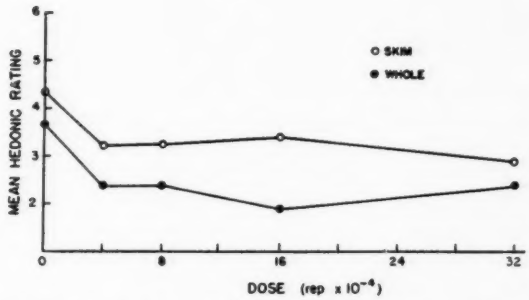

FIG. 4. Mean hedonic ratings of powdered milk irradiated wtih high voltage cathode rays prior to reconstitution.

This observation indicated that the heat treatment necessary for high-temperature, short-time pasteurization did not increase or decrease the sensitivity of these products to the development of off-flavor induced by radiation.

Homogenization of pasteurized whole milk, on the other hand, significantly increased the resistance of the milk to the development of radiation-induced off-flavor. Homogenization has been reported by Krukovsky *(18)* and others to retard or inhibit the development of copper-induced oxidized flavor in milk.

The effect of spray drying and evaporation of whole milk on the recognition threshold dose cannot be attributed to the unit process involved because the proximate composition of these products after reconstitution differed slightly from that of the pasteurized fluid whole milk. For both reconstituted whole milk powder and evaporated milk, the off-flavor recognition threshold dose was higher than that for the pasteurized whole milk.

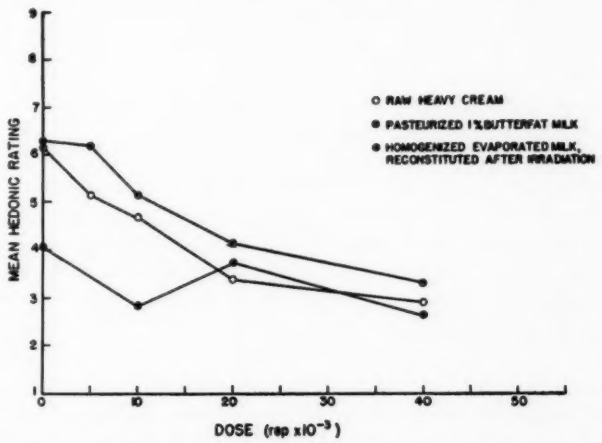

FIG. 5. Mean hedonic ratings of various dairy products irradiated with high voltage cathode rays.

The composition of the dairy product influenced the dose level at which radiation-induced off-flavors were detectable and recognizable (Table 2). The component exerting the greatest influence was the moisture content. The greater the moisture content, the more readily did the product develop objectionable off-flavors. This is best illustrated by comparing the off-flavor recognition threshold doses for powdered skimmilk irradiated *(a)* as a powder prior to reconstitution (RTD = 106,700 rep) and *(b)* as a liquid after reconstitution (RTD = 14,800 rep). In the former case the moisture content was 1.6%, in the latter 90.7%. The increase in concentration or the percentage of total solids thus appears to exhibit a "protective effect" against the production of off-flavor. The influence of solute concentration on the net effect of cathode rays has been described previously by Proctor *et al. (23)*. The relative absence of fat in the skimmilk has also been shown to reduce the susceptibility of this product to off-flavor development upon irradiation.

Fluid skimmilk, milk containing 1% butterfat, and whole milk gave respectively decreasing recognition threshold doses. A significant difference was obtained between the recognition threshold doses for skim and whole milk.

Since an increased fat content appeared to be related to the dose level at which radiation-induced off-flavors could be detected and recognized, the recognition threshold dose was determined for heavy cream containing approximately 44% butterfat. The recognition threshold dose obtained for this cream was 8,020 rep. Since the recognition threshold dose obtained for whole milk was 7,210 rep, it was surmised that this threshold dose for heavy cream would be considerably lower. Actually, this was not the case. One explanation for this reduction in sensitivity may be the protective effect caused by the decrease in moisture content and the increase in total solids. This protective effect is explained by the fact that, although ionizing radiations in the main destroy bacteria by direct hits, these radiations cause the undesirable side reactions indirectly via the free radicals and the activated molecules produced in the solvent. Therefore, the lower the moisture content and/or the greater the complexity of the medium, the less the indirect effect.

Although proteins also have been implicated in the development of off-flavor produced by ionizing radiations, their influence in this study was not estimated.

Many different characterizations of the off-flavor produced in milk by ionizing radiations have been noted. The off-flavor has been variously described as oxidized, rancid, tallowy, chalky, scorched, wet dog, and even as burnt wool. Although the flavor description varies, the area of sensitivity to this flavor has been found to be localized in the posterior section of the oral cavity in the area of the soft palate. The flavor at threshold levels is noticed only upon swallowing and then lingers as an aftertaste.

The use of experienced tasters for detection and recognition of off-flavor in this study undoubtedly resulted in off-flavor threshold levels that may be presumed to be below those of untrained consumers. As previously mentioned, studies by other investigators on the preservation of milk by radiation have indicated that irradiation of milk with 2 m.e.v. X-rays at doses below 73,000 roent-

gens produced no noticeable changes in flavor. The inconsistencies in results may have been due to the differences in the sensitivity of panel members or to differences in the experimental procedures.

SUMMARY AND CONCLUSIONS

The general objectives of this investigation were *(a)* to establish the dose levels at which cathode-ray-induced off-flavor is detectable and recognizable in various dairy products, *(b)* to establish the effect of this off-flavor on the acceptability of the products, *(c)* to determine whether certain preprocessing methods influence the susceptibility of these products to development of radiation-induced off-flavor, and *(d)* to demonstrate whether fat or moisture content has an effect on the off-flavor threshold level.

The threshold doses at which off-flavor induced by high voltage cathode rays was recognizable in cream and various types of whole and skimmilk ranged from approximately 7,000 to 25,000 rep, depending on the product. The acceptability of the irradiated products decreased with increased dose.

Prepasteurization of milk by the high-temperature, short-time method did not significantly alter the susceptibility of milk to radiation-induced off-flavor development.

Homogenization significantly increased the resistance of pasteurized whole milk to the development of off-flavor induced by ionizing radiations.

Both the moisture and the fat content of the milk and cream influenced the threshold dose level at which radiation-induced off-flavors were recognizable. As the moisture content increased, off-flavor production increased. Likewise as the fat content increased, the off-flavor production increased. However, as the total solids increased, the product became less susceptible to flavor change. Therefore, in a product like cream, in which both the fat content and the total solids content are increased, there appears to be a balancing of susceptibility and protection.

The use of mathematical line-fitting and confidence limits on log-probability paper offers a new method of studying significant flavor differences at the threshold level.

This paper points out the need for basic research into the types of changes occurring in foods as a result of treatment with ionizing radiations.

ACKNOWLEDGMENTS

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RADIATION PRESERVATION OF MILK AND MILK PRODUCTS. III. THE THIOBARBITURIC ACID TEST AS A MEANS OF EVALUATING RADIATION-INDUCED CHANGES IN MILK[1]

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Research on the effects of ionizing radiations on milk has been undertaken during the past year with emphasis on the study of the nature of the changes occurring in irradiated milk. From these studies a means of obviating undesirable reactions should be found, and ultimately the result will be the production of a high quality, radiation-sterilized product.

The research reported in this paper was directed toward an evaluation of the usefulness of the thiobarbituric acid test as an objective means of evaluating irradiation-induced changes in milk. The findings of a number of extensive background experiments are summarized here in order to aid in understanding what has been done in this field.

Early in the course of this investigation it was observed that all types of milk (skimmilk, whole homogenized, and evaporated), as well as cream, evidenced off-flavors when irradiated. The organoleptic character and the intensity of these off-flavors and the threshold doses varied to some extent according to product *(2)*. Irradiation of total milk fats, as well as irradiation of solvent extracted residue, produced off-flavors. The off-flavor complex, with the exception of a tallowy constituent, was found to be volatile, and it was possible to condense the off-flavor complex under dry ice when irradiated milk was subjected to vacuum distillation.

A 1,000-ml. Pyrex round-bottom flask with ground joints was fitted with a 250-ml. separatory funnel with drip tip and with a 4-in. distilling column connected through ground glass joints and a vapor line with a dry ice trap. A vacuum of 4-5 mm. Hg was maintained, and 200 ml. of irradiated milk was slowly introduced through the separatory funnel. A water bath of 40° C. was maintained at a level slightly above that of the boiling milk within the flask. This water level was raised as the contents in the flask increased. The ring of warm water prevented undue foaming in the "boiler." The flow of milk was maintained at approximately 5-7 ml. per minute. Distillation was continued for an additional 5 minutes after all the irradiated milk had been added to the flask. Under these conditions, approximately 100 ml. of condensate was recovered from the dry ice trap. The total distillation time was about 45 minutes.

The condensates were shown to contain carbonyls (established by formation of their 2-4 dinitrophenyl hydrozones), and the presence of S-S groups was established by reaction with sodium nitroprusside after reduction with sodium cyanide.

Skimmilk, irradiated with 2×10^6 rep, was subjected to vacuum distillation and stripped of irradiation flavor. The resulting condensate contained a characteristic irradiation flavor reminiscent of wet wool. When this condensate was

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reintroduced into fresh unirradiated skimmilk, an irradiation-like off-flavor was obtained. The same off-flavor complex was found to occur in condensates of irradiated whole milk, with the further addition of a characteristic linseed oil reversion flavor.

Several investigators have studied the relation of thiobarbituric acid (TBA) values at 535 mμ to the organoleptic evaluation of oxidized flavor and have found good correlation of optical density with flavor intensity *(3, 5, 6, 14, 15, 21, 22)*. Accordingly, it was felt that the thiobarbituric acid reaction as described by Kohn and Liversedge *(13)* and Bernheim *et al. (1)* might offer additional objective methods to characterize the irradiation off-flavors in milk.

MATERIALS AND METHODS

The TBA test for milk as explained by Dunkley *(5, 6)* was used in this investigation, with the exception that the volume of extraction solvent was increased from 15 to 20 ml. No correction of optical density for reactive copper was made, since the TBA values were expressed as ΔE values, or the increase in optical density of irradiated milk over the corresponding TBA value of unirradiated milk.

The TBA values of irradiated whole milk and of homogenized milk were found to be identical. Homogenized milk, however, offered advantages because of the greater stability of the samples during storage and during prolonged boiling under vacuum. Therefore, homogenized pasteurized milk obtained from a commercial dairy was used in this study unless otherwise indicated.

Milk was irradiated by high-energy cathode rays produced by a Van de Graaff accelerator at 3 m.e.v. The samples, placed in crystallizing dishes (8 cm. diameter) at a liquid depth of 8 mm., were moved on a conveyor through the electron beam so as to receive the predetermined dose.

RESULTS

TBA reaction of irradiated whole milk. All types of irradiated milk were found to produce positive TBA values. Transmittance in the visible spectrum, however, showed that (at least in the region of the main absorption at 500 mμ) different pigments had been formed. Absorption spectra of TBA pigments derived from homogenized milk irradiated at different dose levels are shown in Figure 1. The absorption at 460 mμ is generally ascribed to sugars and to their degradation products *(9)*. A maximum at 535-538 mμ is considered characteristic for oxidized fats, and because of the proximity of the peak at 500 mμ a shift in the unseparated pigment mixture to 530 mμ would be expected.

Examination of Figure 1 also shows that the relative rate of production of the pigment absorbing at 500 mμ was higher with increasing dose than the rate of the other pigments. The peak at 500 mμ was therefore considered as the absorption characteristic for irradiated milk. When the differences in optical density (as ΔE) at 500 mμ were plotted against dose (Figure 2), the production of TBA reactants occurred as a linear function of dose over a range of 1×10^5

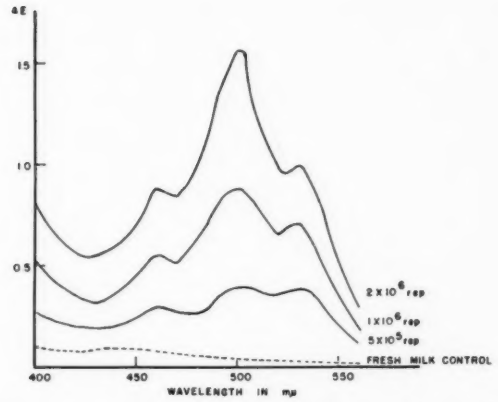

FIG. 1. Absorption spectra of the pigments derived from the reaction of 2-thiobarbituric acid and homogenized milk irradiated with cathode rays. Plot of the difference in optical density (ΔE) between irradiated and control samples. [Note: In the control sample, the ordinate is the optical density (E) itself.]

to 2×10^6 rep. The straight line shown is that calculated by the method of least squares using the mean data at each dose. This function was represented by:

$$y = -0.0093 + 0.0819x$$

When off-flavor components were removed by vacuum distillation, the reconstituted residue gave the same TBA color intensity as the original (undistilled) irradiated milk. The condensates, on the other hand, which displayed characteristic irradiation flavor, gave no TBA coloration. The TBA reactants, therefore, cannot be considered as belonging to the off-flavor complex. However, our observations on the production of TBA reactants absorbing at 535 mμ from irradiated butterfat and from irradiated glycerol would suggest that off-flavors and some part of the TBA reactants might be cleavage products formed concurrently from larger molecular aggregates.

The TBA pigments, however, seemed useful as objective indicators of the intensity of side reactions with milk solutes. A reduction of TBA coloration was observed not only with lower doses of irradiation but also with some of the known technological modifications which minimize side reaction. A decrease in TBA coloration was observed when sodium ascorbate was used as a protective component *(18, 19)* and also when milk (packed in dry ice) was irradiated in the frozen state (Figure 3).

The protective action of sodium ascorbate in preventing the production of off-flavors was found to be greater than the corresponding TBA values indicated, whereas the protective action of freezing was found to be smaller than the corresponding TBA values.

TBA reaction of irradiated milk components. Homogenized milk was lyophilized, freed of lipoidal material, and reconstituted. The combined lipoids were emulsified.

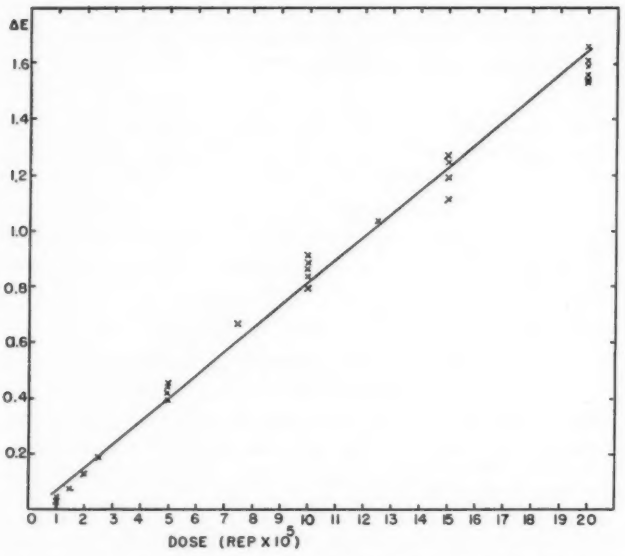

FIG. 2. Thiobarbituric acid reaction of homogenized milk. Plot of difference in optical density at 500 mμ between irradiated milk and control (ΔE) versus irradiation dose.

One thousand-ml. Pyrex round-bottom flasks containing 200-ml. portions of homogenized milk were rotated in alcohol and dry ice so as to form a solid layer of frozen milk on their walls. These flasks were connected to dry ice traps and a vacuum of less than 2 mm. Hg was maintained until, after approximately 8 hours, all moisture had been removed by sublimation. The remaining milk solids were extracted in a Soxhlet apparatus first with ethanol and then with petroleum ether and diethyl ether. Solvent was removed by evaporation under vacuum. The fat-free residue was reconstituted by dispersing 8 g. in 100 ml. distilled water. The combined milk fats were emulsified at 30° C. in a concentration of 4% in water with Bentonite (4%) as a radio-insensitive dispersing agent. A suitable high-speed mixer (Eppenbach Homomixer or Waring blendor) was used for emulsification. To obtain stable emulsions, the material was cooled immediately after emulsification to 10° C. by stirring with a slowly moving laboratory agitator in ice water as a coolant.

Irradiation of the fat free reconstituted residue with a dose of 1×10^6 rep caused intense off-flavor. The absorption spectrum of TBA pigments obtained from this irradiated residue is shown in curve A of Figure 4. The absorption maxima are located at the same wave lengths (460, 500 and 530 mμ) for the irradiated reconstituted residue as for irradiated homogenized milk (Figure 1), although the proportionate amounts of pigment differ.

Irradiation of emulsified total milk fats with 1×10^6 rep of cathode rays resulted in off-flavor development, characterized by flavors of the linseed oil reversion type. The TBA coloration is shown in curve B of Figure 4, which indicates one peak at 535 mμ. Comparison of curves A and B of Figure 4 with Figure 1 shows that the component exhibiting absorption at 535 mμ was not produced from lipoids alone. The component showing absorption at 500 mμ, on the other hand, seems to originate from nonfat constituents only.

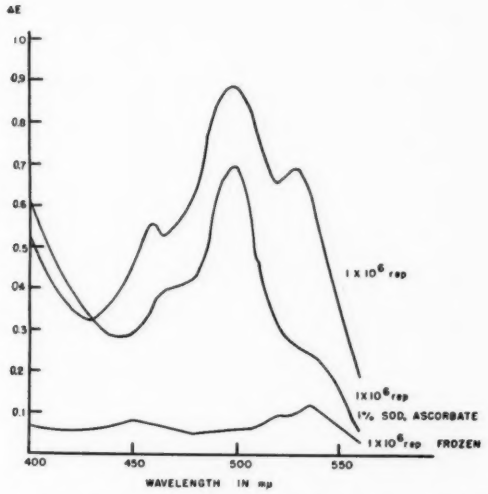

FIG. 3. Thiobarbituric acid reaction of homogenized milk irradiated in the fluid state at approximately 40° F. with and without the addition of sodium ascorbate and also irradiated in the frozen state.

The TBA reactants were found to be water-soluble and seemed to occur only in the water phase. Separation of butterfat from irradiated milk with a nonionic surface active agent and sodium tetraphosphate *(24)* gave a negative TBA reaction in the fat phase.

Demonstration of the dilution effect on irradiated cream is presented in Figure 5. Heavy cream (40% butterfat) and a 10% dilution of heavy cream were irradiated with a dose of 2×10^6 rep cathode rays. After dilution to equal concentration, the TBA reaction showed considerably higher density of the sample irradiated in diluted form. This corresponded generally with the off-flavor development, which was on a very high level in the diluted, irradiated cream.

Chromatographic separation of TBA pigments. The relative rate of light absorption at 500 mμ and at 530-35 mμ was found to change with increasing dose. This was taken as an indication that instead of one pigment with two maxima being produced, the component absorbing at 500 mμ and 530-35 mμ represented several distinctly different groups. (See Figure 1.) It also appeared that at 530-35 mμ the reactant causing the TBA absorption, which had been ascribed to oxidative rancidity of fats, was either also produced by nonfat molecules or was composed of several reactants of different origin. To attempt to clarify some of these questions, chromatographic separation of the pigments was begun.

It was found that acid precipitation and clarification of the resulting whey from irradiated homogenized milk did not affect the quantity of TBA reaction. The use of whey derived from irradiated milk greatly facilitated filtration

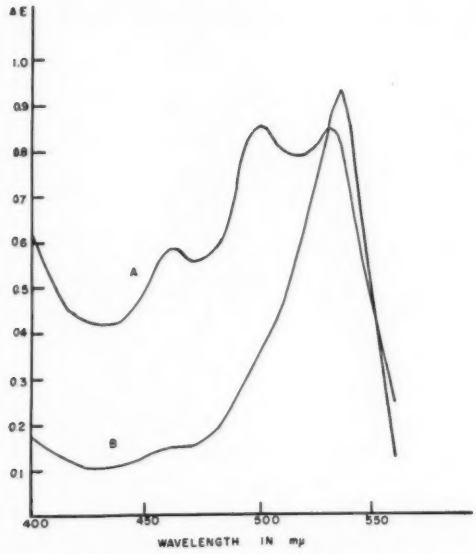

FIG. 4. Thiobarbituric acid reaction of irradiated (1×10^6 rep) reconstituted extracted milk residue (A) and irradiated (1×10^6 rep) re-emulsified total milk fat (B).

through the absorption column. Following is a description of the procedure adopted to separate the pigments on columns.

Irradiated homogenized milk (50 ml.) was brought to 30° C., and 1.3 ml. of a molar solution of H_3PO_4 was added while the milk was stirred to lower its pH to 4.0-4.1. The casein-fat precipitate was removed by centrifuging, and the whey was decanted and clarified by filtration through Hyflo-Supercel. The method of Dunkley *(5, 6)* was then carried out by reacting 10 ml. of clarified whey with 5 ml. of TBA reagent. After being heated in boiling water for 12 minutes and rapidly cooled in ice water, the pigments remained in clear solution. These solutions were chromatographed on columns of 8 mm. diameter, packed with Whatman standard grade cellulose powder *(4)* to a height of 180 mm. The total 15-ml. reaction mixture was transferred to the column, and nitrogen pressure was applied to ensure an approximate flow rate of one drop per second.

The column was washed with acidified water (0.6% H_3PO_4). The use of distilled water was found to cause premature elution of the red pigment, but acidified water allowed the washing out of yellow pigments. The first 15 ml. of filtrate from the cellulose column contained the bulk of unadsorbed pigments and was set aside. Approximately 50 ml. of acidified water was used to wash the remaining yellow and brownish pigments from the paper column.

A red band that showed strong fluorescence in the ultraviolet light remained on the column. A narrow blue band was found above the red, which seems characteristic for irradiated milk, and did not appear in the samples from the unirradiated control. At this time, no efficient eluant has been found to remove this

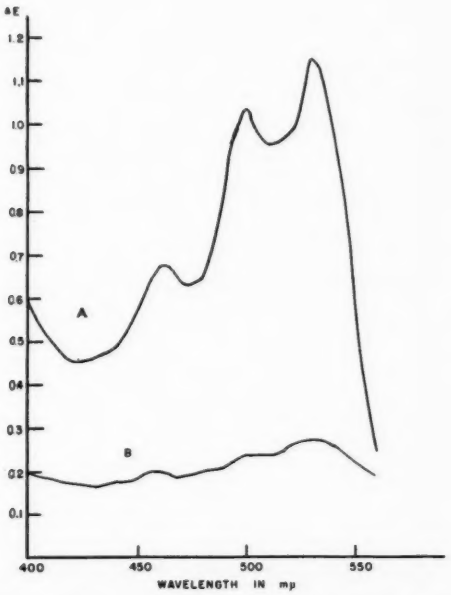

FIG. 5. Thiobarbituric acid reaction of heavy cream. (A) Diluted before irradiation to 4% butterfat; (B) diluted after irradiation to 4% butterfat.

band, but work on this component is being continued. The red band was eluted with 20% aqueous pyridine, and the absorption spectrum in the visible region (Figure 6-A) indicates the presence of only one maximum at 535 mμ.

The filtrate from the paper column was readsorbed on silicic acid–celite (2:1) by use of a column of 12 mm. internal diameter packed to a height of 150 mm. Nitrogen pressure was applied to obtain a flow rate of approximately one drop per 2 seconds. A brownish yellow filtrate remained unadsorbed, and a purple band was left on the column. This was washed with 15 ml. of acidified water (0.6% H_3PO_4) to remove all yellow pigments. This band showed no fluorescence in the ultraviolet light. It was eluted with 20% aqueous pyridine (2 to 3 ml. was sufficient to remove the band), the eluate was diluted to 15 ml. with water, and the pigments were extracted with pyridine-isoamyl alcohol by shaking for 2 minutes and centrifuging. Figure 7-A shows the transmittance of this component, indicating one maximum at 500 mμ.

Chromatographic separation of the TBA pigments from irradiated homogenized milk, from nonfat milk, and from fat-free extracted milk residue gave components absorbing at 500 mμ and 535 mμ. All these 500-mμ components showed similar absorption curves, did not adsorb on cellulose, adsorbed on silicic acid, and did not fluoresce in ultraviolet light. The 535-mμ components also had similar absorption characteristics, showed orange fluorescence in the ultraviolet light, and had similar absorption spectra with one maximum at 535 mμ. Figure

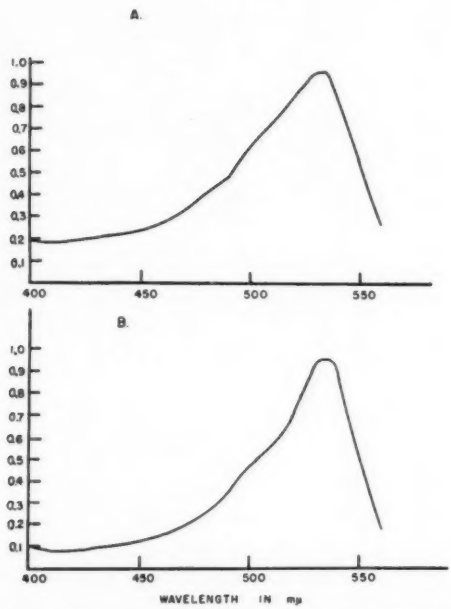

FIG. 6. Thiobarbituric acid component 535 mμ. (A) Separated from irradiated homogenized milk; (B) separated from irradiated fat free milk residue.

6-B presents the 535-mμ component derived from an irradiated fat-free milk residue, which appears similar to the component shown in Figure 6-A.

DISCUSSION

Patton and Kurtz *(15)* found similarity between the pigment produced by reaction of malonaldehyde with TBA and the pigment formed from oxidized milk fat. Shepherd *(23)* and Jennings *et al. (12)*, however, showed that the identical optical behavior of these pigments did not imply common chemical origin and that pyrimidine derivatives gave similar transmittance when they reacted with TBA. Therefore, the similarity of the optical and physical behavior of 535-mμ components, derived from irradiated nonfat constituents as well as from irradiated milk fat emulsions, cannot be taken as a priori proof of identity. Further research will be required to establish the identity and origin of these 535-mμ components.

The production of a TBA pigment with a maximum of 500-510 mμ was reported by Jennings *et al. (12)*, who treated the 528-532 mμ component with excess iodine. It is not known at this time whether the radiation-induced 500-mμ compound is related to this TBA pigment. TBA pigments with identical behavior to radiation-induced 535-mμ were produced from irradiated 2% aqueous glycerol. Irradiation of aqueous solutions of ethylene glycol with cathode rays produced a TBA component identical in its behavior with component 500-mμ (Figure 7-B).

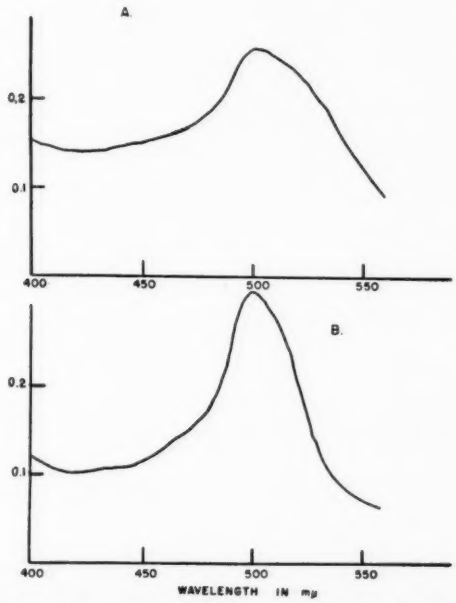

FIG. 7. Thiobarbituric acid component 500 mμ. (A) Separated from irradiated homogenized milk; (B) separated from irradiated ethylene glycol.

The components of irradiated milk responsible for the radiation-induced TBA pigmentation were found to be in a dynamic state. After the irradiation process ceased, the intensity of the TBA reaction decreased with time when irradiated milk was stored at 40° F. This after-effect with irradiated milk is demonstrated in Figure 8. Freezing and storage at –20° F. arrested this reactivity. Irradiated milk stored for 52 days at –20° F. retained 93% of its TBA color intensity. Similar behavior has been observed with respect to the irradiation flavor of milk *(20)*, and it is believed that both phenomena might be caused by condensation or polymerization of carbonyl groups of TBA reactants as well as of off-flavor molecules.

SUMMARY

The TBA reaction was found to be useful as an index of the degree of radiosensitivity of milk. Values of optical density at the main absorption maximum at 500 mμ exhibited a linear function with respect to dose. The values are believed to express relative amounts of milk degradation, in fair agreement with results obtained by measurement of the browning reaction.[2]

Chromatographic separation showed that several groups of pigments were formed. The red pigment absorbing at 535 mμ was produced by all milk com-

[2] The results obtained by measurement of the browning reaction will be reported in the next paper of this series.

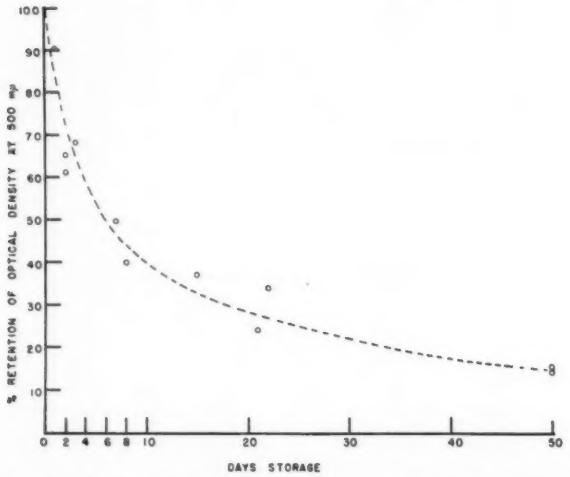

FIG. 8. Thiobarbituric acid reaction of irradiated (2×10^6 rep), homogenized milk during storage at 40° F. for 50 days.

ponents investigated. The component produced by irradiation of emulsified milk fat seemed similar to the component from irradiated fat-free milk residue.

Pigments absorbing at 500 mμ, as well as a heterogeneous group of yellow pigments, were produced by irradiation of all milk components except those from purified fats. The simplest components producing pigments absorbing under similar conditions on irradiation were found to be glycerol and ethylene glycol.

The observations reported herein indicate that the irradiation-induced TBA reactants are not off-flavor components but part of a complex of other irradiation-induced chemical changes. Some of these might occur parallel to off-flavor development.

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KETOSIS IN DAIRY CATTLE. A REVIEW

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Clinical ketosis in ruminants, particularly cattle, is unique in that it is a primary metabolic disorder or syndrome, which is not secondary to any other recognized disease, such as is true of the ketosis often associated with diabetes in humans.

What appears to have been ketosis or acetonemia in dairy cows was described as early as 1849, according to Udall *(137)*. Several case reports were made before 1874 *(27)* describing symptoms which are so typical of what is now often referred to as the nervous type of the disease as to leave little doubt that at least some were identical to the cases of bovine ketosis which are now so prevalent. The condition was referred to as mania puerperalis. Chloral hydrate was employed in treatment.

Sjollema and Van Der Zande *(127)* were the first to present data showing abnormally high levels of acetone bodies in the blood and urine of cows exhibiting symptoms of ketosis. Their description of the symptoms and conditions associated with the occurrence of ketosis can hardly be improved upon today. For example, they reported that fat, high-producing cows were more susceptible and that the conditions usually occurred 7 to 10 days postpartum. Also observed were rapid loss of body weight, lack of appetite, dry feces, rapid decrease in milk production and, in many cases, nervous disturbances. There was a marked improvement when cows were turned out to pasture. They also reported that the liver of a cow that died with ketosis was yellow and enlarged and exhibited fatty degeneration. Hutyra and Marek *(45)* reported that the disease is most frequently observed in cows after the third to sixth calf and that it usually makes its appearance when the animals are stabled. Repeated attacks were observed following subsequent parturitions. Hupka *(43)* first demonstrated the hypoglycemia associated with ketosis in cows and noted that sugar administration was beneficial. Stinson in 1929 *(130)* reported that the subcutaneous injection of glucose was a very effective treatment for ketosis, which he referred to as postparturient dyspepsia.

Thus, the basic concepts of hypoglycemia and ketonemia and the beneficial effect of glucose therapy were recognized by 1929 and, although numerous reports on bovine ketosis have appeared in the literature subsequent to these early reports, it has been only during the past decade that appreciable additional advances have been made.

In this review particular attention will be given to the more recent findings in the field of bovine ketosis. The term ketosis, unless otherwise qualified, will be used in reference to primary or clinical ketosis, as is further defined in the section on diagnosis. The terms acetone bodies and ketone bodies will be used interchangeably.

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INCIDENCE

Bovine ketosis is of common occurrence, being one of the major disease problems in all areas of the world where there are any appreciable number of dairy cattle. It has been reported that in some herds from 15 to 40% of the cows exhibit ketosis *(87)*. Shaw *(100)* reported a study in a herd in which 12 of the 16 cows that calved during a 3-month period developed ketosis. Nichols *(67)* conducted a survey among registered large animal practitioners in Wisconsin for the year 1951, from which he estimated that 60,000 cows were treated for ketosis in Wisconsin in that year. It may be estimated roughly that 1,000,000 cows per year require treatment for ketosis in the United States. Numerous reports by Sjollema and Van Der Zande *(127)*, Hupka *(43)*, Messervy *(63)*, and McIntosh *(61)* indicate that ketosis is a common disease of dairy cattle in Europe and Canada.

Reports by Allcroft *(1)*, Carlström *(13)*, Shaw *et al. (115)*, and others are in agreement with the conclusion of early workers *(36, 45)* that ketosis usually occurs from a few days to a few weeks postpartum. The highest incidence appears to occur about 3 weeks postpartum; only a limited number of cases occur during the prepartal period and after two or more months postpartum. Since ketosis is often difficult to diagnose, it is quite likely that many such cases may not represent primary ketosis *(115)*.

In many areas ketosis appears to be most prevalent during the winter or stall-feeding period. It is somewhat difficult to evaluate the reports, since data are not available to show to what extent the seasonal effects are associated with the calving season. Hutyra and Marek *(45)* observed that ketosis usually makes its appearance when animals are stabled. Sjollema and Van Der Zande *(127)* stated that cows recovered from ketosis when put on pasture. However, Allcroft *(1)* compiled data showing that there was a rather uniform occurrence of ketosis throughout the year in Jersey, whereas in the British Isles there appeared to be a seasonal occurrence, with the bulk of the cases occurring in the winter period.

In a continuous study of bovine ketosis by the author during a 14-year period in the states of Connecticut, New York, Delaware, Pennsylvania, Virginia, and Maryland, the incidence of ketosis has been found to be so seasonal that work on field cases is suspended during the summer months. Seasonal calving and the apparent antiketogenic effect of pasture feeding *(123)* appear to be the major factors controlling seasonal incidence in this area. Records obtained in recent studies by Shaw *et al. (111)* in the Los Angeles area, in which calving is rather uniform throughout the year, showed that the incidence of ketosis was also relatively uniform from month to month and appeared to be unaffected by pasture feeding. Although only one-third of the cows that calved during the 3-month period of study were animals recently shipped into the area, 57% of the cases of ketosis occurred in this group.

Hutyra and Marek in 1926 *(45)* stated that ketosis is most frequently observed after the third to sixth calf. Reports by Hayes *(36)*, Fincher *(25)*,

Allcroft *(1)*, and others confirm this belief and show that an appreciable number of cases may also occur at the first calving. Hayes considers this to be different from parturient paresis, which occurs more frequently in older cows. The reason for the age aspect is not known, but it appears to favor the view that ketosis is of metabolic origin.

Although there is some evidence that ketosis may be an inherited characteristic *(33)*, this does not appear to be of primary importance in most herds. Numerous observations have been made on large herds with a high incidence of ketosis in which cows in the same herd with markedly different ancestry developed ketosis. For example, in a herd studied by Shaw *(100)* in which 12 of the 16 cows which calved during a 3-month period developed ketosis, at least three completely different lines of breeding were involved.

Heredity may be involved, however, in the sense that cows bred for high milk production are more susceptible to ketosis, although there is no precise relationship between milk production and the incidence of ketosis, since in many high-producing herds ketosis is not a problem. The fact that many cows develop ketosis year after year suggests that the genetic make-up of these animals may render them less resistant to the causative factors. More information is needed, since the evidence at the present time is insufficient to establish whether the succeeding generations of such animals are more susceptible to the development of ketosis than the average.

The possible relation of breed to the incidence of ketosis is not entirely clear, although it does appear that no one breed can be singled out. Fincher *(25)* reported that in 131 animals with abnormal amounts of ketone bodies in the urine, nearly all breeds were represented, the highest incidence being among Guernseys and Holsteins, and the lowest among Ayrshires. As no population figures were given, it is not possible to establish whether there was a true breed difference. MacKay *(57)* reported that the disease is found with equal frequency in Shorthorns, Friesians, and Ayrshires. Kingman *et al. (50)* reported that ketosis occurs frequently in the heavier milking beef breeds.

It is generally agreed that the economic loss caused by ketosis is high because of loss of milk, loss of body weight, cost of medication, and the disposal of many animals that have had repeated attacks of the disease *(19, 86, 127)*. Milk production may decrease by more than 50%. The body weight loss will be somewhat dependent upon the severity of ketosis and the time required for recovery but may be as much as 200 lb. in 30 days *(20)*. In a recent review *(2)* it was estimated that the annual loss to the dairy industry in the United States may exceed ten million dollars. Considering the number of cases per year in the United States as one million, it does not appear that this is an exaggeration.

DIAGNOSIS

Bovine ketosis is often difficult to diagnose, yet an accurate diagnosis is an extremely important factor in the effective treatment of this condition *(51, 115)*. Unfortunately, ketosis is frequently accompanied by various complications, such as metritis, retained placenta, nephritis, and other abnormalities. In such cases,

it is difficult to determine whether the ketosis is primary or secondary, that is, whether the ketosis is the true syndrome of deranged carbohydrate metabolism in which the complication may be either coincidental or a major stress factor, or whether the ketosis may be merely that of fasting ketosis, or ketonemia and ketonuria due to certain pathologic conditions other than the true ketotic syndrome. In this discussion the term "primary ketosis" will be used to designate the clinical ketotic syndrome in which complications may or may not exist. The term "secondary ketosis" will be used to designate conditions in which an excess of ketones is present, as the result of fasting or pathologic or metabolic disturbances other than those associated with the true ketotic syndrome. In the final analysis, a response to some of the most effective treatments may have to be used to differentiate between primary and secondary ketosis, since some definite and perhaps temporary response usually will be noted in primary ketosis, even though complications exist.

It is conceivable that, from time to time, certain abnormalities, such as metritis, may constitute the final stress factor responsible for the development of primary ketosis. On the other hand, many cows with metritis and other infections have poor appetites and exhibit a fasting ketosis, rather than primary ketosis, from time to time, so that it is often difficult to differentiate between primary and secondary ketosis. Thus, a positive urine qualitative test does not necessarily mean that a cow has primary ketosis. A negative urine qualitative test, however, rules out ketosis.

It becomes essential, therefore, to pay particular attention to the symptoms, as well as possible complications. A poor appetite, loss in milk production, and either lethargy or high excitability are usually in evidence. These symptoms may be accompanied by paresis or incoordination. A rapid emaciation will be observed in cows that are not treated promptly or that do not recover quickly after treatment.

A low level of blood glucose and a high level of blood or urine ketone bodies are not sufficient evidence alone for a correct diagnosis of ketosis. It has been pointed out that underfeeding will produce a hypoglycemia and ketonemia but will not produce the symptoms typical of primary ketosis. In fact, the blood glucose of cows maintained on a low energy intake postpartum was decreased in many cases to values much lower than that usually observed in primary ketosis *(120)*. In these studies the blood and urine ketone bodies did not increase to the levels often observed in primary ketosis but were sufficiently high to give a positive urine qualitative test and to make it impossible to differentiate from primary ketosis on the basis of the qualitative test alone. A failure to get some definite response to a glucose injection is good evidence that ketosis is not the primary ailment. A failure to get a good response to an adequate dose of one of the more effective glucocorticoids or ACTH is even better evidence that one is not dealing with primary ketosis. For example, in a study in the Los Angeles area *(111)* in the summer of 1954, of a total of 97 ketotic cows treated with varying dosages of glucocorticoids and ACTH, less than 5% failed to show a definite response to as little as 0.5 g. of hydrocortisone.

In screening the effectiveness of various substances for the treatment of ketosis, it is advisable to use only animals which exhibit definite hypoglycemia and ketonemia or ketonuria and typical symptoms of ketosis, uncomplicated by various abnormalities, even though it is recognized that a normal level of blood glucose may exist from time to time and that primary ketosis is frequently associated with various abnormalities.

Approximately 30% of some 300 cases diagnosed as ketosis in the field were observed to be secondary rather than primary *(115)*. This emphasizes the importance of diagnosis in the treatment of ketosis, since treatments generally used for primary ketosis would not be expected to be particularly effective in such cases.

METABOLIC ASPECTS OF BOVINE KETOSIS

1. *Blood glucose and ketone bodies.* Practically all researchers who have studied bovine ketosis have concluded that hypoglycemia and ketonemia usually exist when the signs of ketosis are observed. In 1943 Shaw *(100)* reported, for the first time, the changes in blood glucose and acetone bodies prior to, during, and after the development of ketosis. In one animal the signs of ketosis were not observed until the ninth day postpartum, although the blood glucose became progressively lower from the third day on. There was a gradual increase in blood acetone bodies to a high level by the ninth day, when the cow exhibited the first signs of inappetence and paresis. Another cow exhibited signs of marked incoordination 12 hours after parturition, during which time blood glucose decreased rapidly but blood ketones were still normal. No further signs of ketosis were observed until the fifth day, when the cow exhibited inappetence, lethargy, incoordination, marked hypoglycemia and ketonemia. Both of the above cows had developed ketosis for several years consecutively and required repeated injections of glucose each year to effect a cure. In these two cases the feed consumed was weighed prior to and during the development of ketosis. In both cases the caloric intake was equal to the caloric requirements, thus showing that the ketosis was not due to a lowered feed intake.

Although hypoglycemia is usually observed in cows exhibiting ketosis, clinical ketosis has been observed in cows with blood glucose values in the normal range *(5, 86)*. In studies at Maryland during the winter of 1954, out of 95 cases of ketosis, 17 had blood glucose values of 38 mg. % or above, marked ketonuria, typical signs of ketosis, and recovered rapidly after the use of glucocorticoids, thus showing rather conclusively that these cases represented primary ketosis. One cow exhibited a blood glucose value of 45 mg. % when the first symptoms of ketosis were observed and values of 35 and 28 on the succeeding 2 days and recovered promptly after treatment with hydrocortisone, thus indicating that the clinical signs of ketosis were not due, fundamentally, to a simple hypoglycemia.

Forbes *(28)* observed hypoglycemia and ketonemia in cows receiving half rations after parturition for as long as 26 days. Although the typical signs of

ketosis were not obtained, he concluded that clinical ketosis is essentially a fasting ketosis. However, as was noted above, Shaw *(100)* observed marked signs of primary ketosis in cows receiving an adequate caloric intake prior to and during the development of ketosis. In later studies it was observed that a low level of energy intake immediately postpartum resulted in marked hypoglycemia and moderate ketonemia, but no symptoms of ketosis *(102, 120)*.

Hupka *(43)* appears to have been the first to report hypoglycemia in bovine ketosis and to suggest that hypoglycemia is the essential and primary disturbance. He stated that this concept is supported by the knowledge that symptoms soon disappear after sugar is given. Sampson and Hayden *(90)* concluded that acetonemia of milch cows was caused by a disturbance in carbohydrate metabolism, due either to a lack of sufficient carbohydrate or carbohydrate-forming material in the ration or to an insufficient supply of carbohydrate stored in the liver. In studies with ketotic cows, Shaw *(100)* demonstrated hypoglycemia, low liver glycogen, normal glucose tolerance curves, and normal uptake of glucose by the mammary gland. From these observations it was concluded that the development of ketosis is due to faulty carbohydrate metabolism at a time when the demands for carbohydrates are greatest and in which the inability to maintain a normal level of liver glycogen plays a major role. Johnson *(48)* has recently restated the belief that a lack of sufficient glucose brings about the disturbance. That a lack of blood glucose per se, however, is not the principal cause of primary ketosis is indicated by the fact that cows frequently exhibit clinical ketosis associated with a normal to high level of blood glucose and that levels of blood glucose as low or lower than those commonly observed in primary ketosis have been obtained by partial fasting immediately postpartum but did not result in clinical ketosis.

2. *Glucose metabolism.* In view of the fact that many ketotic cows exhibit a good response to intravenous injections of glucose while others do not, there was some question as to whether cows with ketosis could utilize glucose normally. Glucose tolerance studies were made by Shaw *(100)* on two cows with primary ketosis and a normal cow during the early postpartal period. The two cows with ketosis were resistant to glucose treatment and had developed ketosis for several years consecutively. The glucose tolerance curves were the same for the ketotic cows as for the normal cow and similar to glucose tolerance curves on normal cows later reported by Bell and Jones *(3)*. It is particularly interesting to note that, after the intravenous injection of glucose into ketotic cows, the blood glucose decreased within 2 hours to a level somewhat above the previous low level, but considerably below the normal level. These results were confirmed more recently by Holmes *(39)*. It appears, therefore, that the cause of bovine ketosis is not an inability of the animal to utilize glucose.

One of the etiologic factors that may be of considerable importance in the development of bovine ketosis, which has been overlooked in the past, is the relatively large amount of glucose taken up by the lactating mammary gland. By the use of arteriovenous studies, Shaw *(100)* demonstrated that the glucose uptake by the ketotic cow was of the same order as that of the normal cow. Nine

ketotic cows, with an average arterial glucose level of 27.3 mg. per 100 ml. of blood (mg. %), showed an average glucose utilization of 9.9 mg. %, whereas 13 normal cows with an average arterial glucose level of 45.8 mg. % exhibited an average uptake by the mammary gland of 10.2 mg. %. Thus, it can be seen that relatively large amounts of blood glucose continue to be taken up by the mammary gland of the ketotic cow, even though the glucose content of the blood is considerably below normal. By use of the arteriovenous technique, Shaw *et al. (122)* demonstrated that all of the milk lactose could be accounted for by the amount of glucose taken up by the mammary gland and that little glucose was available for other purposes. More recently Dimant *et al. (15)* demonstrated by the use of radioactive glucose that all of the glucose taken up by the perfused mammary gland was converted into milk lactose. On the basis of 500 volumes of blood per unit volume of milk *(122)* it can be shown that the mammary gland of a cow producing 25 kg. of milk will use in excess of 1,000 g. of glucose. Thus, considerable glucose is required for lactose synthesis.

Using information gained by research on other animals, Roberts and Dye *(79)* calculated that a 1,100-lb. cow should require about 1,500 g. of glucose daily. The relatively large glucose utilization by the mammary gland was not taken into account in this calculation. Also, the glucose requirements of the other tissues of the cow are probably different from those of nonruminants because of the relatively large amounts of acetate and butyrate, which are absorbed from the rumen of the cow and which may be expected to have a sparing effect upon glucose utilization. For example, Drury and Wick *(17, 18)* showed that acetate and β-hydroxybutyrate both decrease glucose oxidation in the rabbit. Butyric acid is converted to ketone bodies by the rumen epithelium and appears in the blood of cows as β-hydroxybutyric acid *(70, 135)*.

It is well known that the liver is the principal source of blood sugar in the fasted monogastric animal. Since the blood sugar level of the monogastric animal is well maintained during relatively long periods of fasting, it is apparent that the blood sugar of the fasted animal must be derived from stored carbohydrate or noncarbohydrate precursors. Carbohydrate is stored in the form of glycogen, and the noncarbohydrate precursors are protein and fat. Although appreciable amounts of glucose are absorbed, as such, from food or from breakdown products of food in the monogastric animal, very little exogenous glucose is available for the ruminant *(60, 75, 91)*. It is important to recognize, therefore, that very little glucose is absorbed from the digestive tract of the ruminant. It is evident that most of the digested carbohydrates and some of the amino acids are converted to volatile fatty acids by the microorganisms of the rumen *(16, 125)* and that very little glucose is absorbed, as such, except when unusually large amounts are administered orally *(123)*.

Since propionic acid is a glycogen former and is produced in the rumen in appreciable amounts, it may be assumed to be one of the important precursors of blood glucose for the ruminant. Among various workers there has been disagreement as to the relative rates of absorption from the rumen of the three volatile acids, acetic, butyric, and propionic. A study by Pfander and Phillip-

son *(73)* demonstrated that the quantities of the volatile acids absorbed from the rumen were in the decreasing order of acetic, butyric, and propionic acids. The mean absorption rates were: acetic 32, propionic 18, and butyric 23 meq. per hour. By assuming that the absorption of these acids in the cow is ten times that in sheep, it is possible to make a rather rough calculation of the amount of blood glucose that may be derived from the propionic acid produced in the rumen. During a period of 24 hours it can thus be estimated that the cow may obtain somewhat in excess of 800 g. of glucose from propionic acid absorbed from the rumen. As indicated above, this would hardly be enough to provide for the glucose requirements of the mammary gland of a good producing cow. For such cows, it appears that additional glucose must be obtained from other sources.

From the above calculations it would appear that gluconeogenesis from protein and fat may be extremely important in the high-producing cow and that propionic acid is probably not the only major source of blood sugar. Thus, a decrease in the production of propionic acid, or possibly lactic acid, faulty gluconeogenesis from protein and fat, or a decrease in the production of the glucose sparing substances, acetic and/or butyric acid (precursor of blood β-hydroxybutyrate) could either decrease glucose production or increase glucose requirements. However, the relatively high level of β-hydroxybutyric acid in the blood of ketotic cows is good evidence that bovine ketosis is not due to a decreased production of butyric acid by the rumen, since rumen butyric acid is utilized by the body via β-hydroxybutyric acid.

As yet, no one has shown that there is a decrease in the production of propionic acid or of acetic or butyric acids by the rumen of the cow prior to the onset of ketosis. That hypoglycemia per se is not the cause of ketosis is suggested by the work of Forbes *(28)* and more precisely by Shaw and Leffel *(120)* and Leffel *(56)*. In the latter studies a marked hypoglycemia was produced by maintaining cows on a low caloric intake immediately postpartum. No symptoms of primary ketosis were observed, even though the blood glucose frequently decreased to levels considerably lower than those observed in most cases of primary ketosis. The degree of fasting in the latter studies was considerably more severe than that usually observed preceding the development of primary ketosis under field conditions. Any theory relating to the etiology of ketosis must explain why fasting during the early postpartal period produces a marked hypoglycemia but does not produce primary ketosis. Likewise, the fact that the intravenous injection of glucose or the oral administration of various glucose precursors, such as propionate or lactate, and glucose sparing substances, such as acetic acid, often results in a marked improvement of the cow with primary ketosis cannot be accepted as evidence that a deficiency of any one of these substances is the cause of ketosis. Similarly, the prevention of ketosis by the administration of glucose precursors would not constitute evidence that the ketosis was due to a deficiency of one or more of these substances in the diet. It is difficult to avoid the conclusion that a faulty gluconeogenesis is one of the major etiologic factors and that the curative effects of the administration of glucose, glucose precursors, or glucose-sparing substances is merely that of providing a new source of glucose,

which, in the normal cow, is ordinarily provided by gluconeogenesis. As will be noted later, this could be associated with an adrenal disfunction.

From some of the above considerations and from observations by Shaw and Leffel *(120)* and Leffel *(56)*, there appears to be a possible explanation as to why it is so much easier to produce hypoglycemia and ketonemia in the early postpartal period than during the later part of the lactation period. It was observed that a low caloric intake during the early postpartal period did not result in the marked decrease in milk production invariably observed when cows were maintained on a low caloric intake during the later part of the lactation period. It is obvious that the stimulation for milk production during the very early part of the lactation period is such that various body reserves are called upon during this period for the secretion of milk, whereas during the later part of the lactation period this marked stimulation does not exist. Thus, milk production of cows on a low energy intake continues at a rather high level during the early part of the lactation period and, among other things, the glucose requirements of the mammary gland remain high at a time when the exogenous sources of glucose are reduced. Cows fasted during the later part of the lactation period, however, decrease sharply in milk production and thus the glucose requirements of the mammary gland are lessened materially.

It appears entirely possible that the amount of glucose produced by gluconeogenesis in the high-producing cow is considerably greater than that derived from exogenous sources. If such is the case, faulty gluconeogenesis would result in a severe tax on the animal because the mammary gland continues to utilize relatively large quantities of glucose, resulting in an even greater glucose deficit for the other tissues of the body. Although considerable quantitative information is now available on glucose utilization by the lactating mammary gland of the cow, there is a dearth of information on the glucose requirements of the other tissues of the body of the lactating cow. This is a fruitful field for research.

3. *Glycogen and lipids.* To estimate the liver glycogen of normal and ketotic cows, Shaw *(100)* measured the increase in blood glucose after the intravenous injection of epinephrin. In normal cows the blood glucose increase persisted for an hour or more, whereas in ketotic cows the blood glucose increased slightly and returned to normal within 20 minutes. In these studies hypoglycemia, ketonemia, and ketonuria were observed in several cows with varying signs of ketosis within a few days postpartum, even though the caloric intake was maintained at a relatively high level. In one such animal, in which a marked hypoglycemia and ketonuria developed within 4 days postpartum, the apparent liver glycogen was very low one day prepartum and one day postpartum. The blood glucose was normal on the day preceding and the day after parturition, but low on the fourth day postpartum. In the same herd a normal cow that maintained a normal blood glucose postpartum and did not develop ketosis exhibited a rise in blood glucose of approximately 1 hour duration on the day postpartum after the injection of epinephrin.

Additional information on the liver glycogen and liver fat of ketotic, normal, and fasted cows (early lactation) was reported by Shaw and Leffel *(120)* and

Leffel *(56)*. Blood glucose and acetone bodies were also recorded. The liver samples were obtained by biopsy. The average liver glycogen and blood glucose values for the normal cows on the higher caloric intake were relatively high, both prepartum and postpartum. The average levels for liver fat and blood acetone bodies were relatively low. In contrast, cows with either primary ketosis or fasting ketosis exhibited low levels of liver glycogen and blood glucose and relatively high levels of liver fat and blood acetone bodies. In these studies, the liver fat of cows during the early stages of ketosis was frequently normal, thus suggesting that the fatty livers often observed in cows with ketosis are probably due to fasting. Low levels of liver glycogen were always observed in cows on a low caloric intake, whereas in cows with primary ketosis there was more variation. Low levels of liver glycogen were observed in two cows during the early stages of ketosis, suggesting that the low levels of liver glycogen of ketotic cows is not due, necessarily, to fasting. Low blood glucose values were always observed in cows on a low caloric intake postpartum. It was also noted that the level of blood acetone bodies of cows with ketosis was usually considerably higher than that of fasted cows at a similar level of blood glucose.

Saarinen and Shaw *(83, 84, 85)* observed that the blood lipids of ketotic cows differed from those of normal cows. Changes of a similar nature were observed in cows on a low caloric intake postpartum, indicating that the differences observed in the blood lipids of ketotic cows were due to an insufficient caloric intake. The increased fat content of the adrenals of ketotic cows was reproduced by maintaining normal cows on a low caloric intake immediately postpartum. The cholesterol content of the adrenals of ketotic cows was about the same as that of normal cows. The ascorbic acid content of the adrenals of ketotic cows was somewhat low, but this was duplicated by withholding feed from normal cows. The flabbiness and hypertrophy observed in adrenals of ketotic cows was not reproduced by withholding feed from normal cows in the early postpartal period. The enlargement of the adrenals of ketotic cows could not be explained on the basis of the water or fat content of these glands.

4. *Ketone body metabolism.* Ketonemia has been associated with the syndrome known as bovine ketosis ever since Sjollema and Van Der Zande *(127)* demonstrated the presence of abnormally high levels of ketone bodies in the blood and urine of cows exhibiting symptoms of ketosis. The ketone bodies, β-hydroxybutyric acid and acetoacetic acid, are found in traces in the blood of normal monogastric animals and are excreted in the urine in very small amounts. In the ruminant, however, there are appreciable amounts of ketone bodies in the blood and urine of normal animals *(53, 89, 90)*.

Numerous studies have demonstrated conclusively that the principal source of ketone bodies in the nonruminant is the fats *(44)*, although it is recognized that metabolic pathways exist for the transformation of almost any substance to ketone bodies *(4)*. The fasting of a normal person for several days results in an increase in blood ketone bodies and the excretion in the urine of as much as 10 g. per day. In diabetes, several hundred grams of ketone bodies may be excreted per day. The normal bovine, on the other hand, has appreciable amounts

of ketone bodies in the blood, usually varying between 2 and 4 mg. per 100 ml. of blood with a maximum, for cows adequately fed, not greater than 6 to 7 *(5, 54, 90)*. Fasting the cow during the early postpartal period may result in increases of the blood ketones to as high as 25 or 30 mg. per 100 ml. *(28, 102, 120)*. Rather severe fasting is required in order to increase the blood ketone bodies above these levels. Considerable importance may be attached to the fact that cows with primary ketosis, however, usually exhibit much higher levels of blood ketones than fasted cows, although the degree of hypoglycemia is often not as marked as that observed under fasting conditions *(120)*. Thin and Robertson *(135)* have also noted that the intense degree of ketonemia in ketotic cows is often out of proportion to the reduction in food consumption. Since bovine ketosis is observed, from time to time, in cows which have a normal level of blood glucose, the question may be raised as to whether bovine ketosis is due to a lack of carbohydrate or to an impairment in the utilization of carbohydrate. The fact that glucose tolerance curves of ketotic cows are normal suggests that there is no impairment of the utilization of carbohydrate. Thus, although there are some parallels between the ketosis of diabetes and bovine ketosis, there are also some marked dissimilarities.

It has been appreciated for some time that the rumen is the source of the relatively large amounts of ketone bodies in the blood of normal ruminants. It is significant that as early as 1933 Brouwer and Dykstra *(8)* demonstrated acetonemia, but not clinical symptoms of ketosis, when 30 kg. of grass silage containing 1.7 to 2.0% butyric acid was fed. Knodt *et al. (54)* observed that the blood acetone bodies of calves were very low during the first 2 months of life. Very little β-hydroxybutyric acid was observed in the blood of the calf during this period. After 2 months there was an increase in both the blood and urine ketone bodies, due primarily to an increase in β-hydroxybutyric acid. Since the gradual increase in the ketone bodies of the calf paralleled roughly the development of the rumen, it was suggested that the rumen was responsible for the relatively large concentration of ketone bodies in the blood and urine of the bovine. Knodt *et al. (52)* also observed an increase in blood ketone bodies within 3 hours after feeding, the greatest increase being obtained when grass silage was fed. The ketone body increase in both blood and urine was observed to be due to β-hydroxybutyric acid, primarily. It was proposed that the increased production of ketone bodies of cows after the feeding of grass silage was due to the volatile acids in the silage. Schultz and Smith *(95)* observed that butyric acid was ketogenic in ruminants and produced a severe decline in blood glucose.

Recent findings have helped to clarify the picture. Pennington *(70)* demonstrated that the rumen epithelial tissue of sheep converted butyric acid to ketone bodies, mainly acetoacetic acid. Small amounts of ketone bodies were formed also from acetic acid but not from propionic acid. It appears, therefore, that the rumen is the major source of the blood ketone bodies of the fed animal. By using a new method for the fractionation of acetone bodies, it was found that β-hydroxybutyric acid is the only ketone in the blood of normal healthy cows *(134, 135)*. Thus, it is apparent that the acetoacetic acid produced from butyric

and acetic acids by rumen epithelial tissue is converted rapidly into β-hydroxybutyric acid.

Thin and Robertson *(135)* reported that the blood ketone bodies of cows with ketosis consisted primarily of acetone and β-hydroxybutyric acid, some acetoacetic acid, and an appreciable amount of isopropanol. It was suggested that acetone and β-hydroxybutyric acid are formed from acetoacetic acid. Since the isopropanol concentration was greater in the rumen than in the blood, they suggested that it was produced in the rumen from acetone, which diffuses in from the blood stream. Observing that the isopropanol concentration in the blood and rumen of cows with primary ketosis was greater than that of cows with starvation ketosis, they suggested that the formation of isopropanol may play an important part in the development of ketosis. The possible significance of isopropanol is not apparent at the present time.

There is some confusion about the significance of abnormal amounts of ketones in the blood of cows. That they are not the cause of the symptoms of ketosis is shown by the fact that the ketonemia produced by the fasting of cows immediately postpartum does not result in primary ketosis, and by the studies of Shaw *(100)* in which symptoms of ketosis were observed very early in the development of this condition before the blood ketones were appreciably above normal. Although acidosis has been reported in cows with ketosis, it appears that it is not an important complication except in rather protracted cases *(5, 90, 126, 131)*. Blood ketones in cows with ketosis may range from as low as 10 to 15 mg. % to values in excess of 80 mg. %. The lower values are sometimes confused with fasting ketosis.

There are two important sites of ketone body formation in the ruminant: the rumen and the liver. In primary ketosis, however, it appears that the liver is the site of formation of the increased production of ketone bodies. The same can be assumed to be true for cows with fasting ketosis. Other tissues form ketones but utilize them at a faster rate than they are formed. It is generally recognized that the ketone bodies arise through condensation of acetyl groups, which are produced either by successive beta oxidation of fatty acids or by oxidative decarboxylation of pyruvic acid, the latter undoubtedly playing a minor role in the animal. Johnson *(48)* suggested that bovine ketosis may be due to a shortage of oxaloacetic acid. It is accepted that the availability of oxaloacetate can regulate ketogenesis by providing a pathway for the utilization of acetyl groups through the citric acid cycle. While admitting that there can be no doubt that oxaloacetate plays some part in the ketogenic process, Weinhouse *(140)* points out that there are relatively unlimited sources of oxaloacetate available in the form of dietary amino acids, so that it is difficult to see how an impairment in carbohydrate oxidation would make oxaloacetate unavailable to the cell, particularly since only extremely small amounts are necessary to produce an antiketogenic effect. At any rate, there is no evidence at the present time of an oxaloacetate deficiency in ketotic cows or in other ketotic animals.

That bovine ketosis is not due simply to a lack of carbohydrate in the body is indicated by the fact that fasting in the early prepartal period produces a

marked hypoglycemia but only a mild ketonemia, whereas in primary ketosis a much more marked ketonemia may often be observed at only moderately low levels of blood glucose and at higher levels of food intake.

Wick and Drury *(142)* and others have demonstrated that the utilization of ketones is not impaired in conditions which are associated with ketosis. This appears to be true, also, in bovine ketosis. For example, it was shown that the lactating mammary gland of the normal cow utilizes approximately 2 mg. % of β-hydroxybutyric acid from the blood traversing the gland, whereas the active mammary gland of the ketotic cow utilizes approximately twice this amount, presumably because of the relatively high concentration in the blood *(99, 119)*. Recently it has been shown by the use of radioactive β-hydroxybutyrate that this substance is one of the principal precursors of the short-chain fatty acids of milk fat *(55)*, and a competition has been shown to exist between acetate and β-hydroxybutyrate for this, and perhaps for other purposes, with the mammary gland showing a preference for acetate. Both acetic and butyric acids are used for the synthesis of milk fat, acetate being used directly, whereas butyrate is first converted into acetoacetic acid in passing through the wall of the rumen and then into β-hydroxybutyric acid. It is apparent, therefore, that β-hydroxybutyric acid is an important metabolite in both normal and ketotic cows.

5. *Role of the rumen acids.* In a review of ruminant nutrition in 1939, Marston *(58)* summarized evidence to indicate an extraordinary intermediate metabolism in ruminants in which short-chain volatile fatty acids play a major role. Since Marston's review, numerous studies have been conducted which have shown short-chain volatile fatty acids are significant end-products of bacterial fermentations within the rumen. Lactic acid is also produced in the rumen in significant amounts under certain conditions. For example, Phillipson and McAnally *(75)* observed lactic acid in the rumen after the feeding of glucose. The formation of lactic acid in the rumen was also observed by Phillipson *(74)* when flaked maize was fed. Hungate *et al. (42)* also observed the production of lactic acid in the rumen after the feeding of glucose or changing abruptly from a diet of hay to grain. Earlier, Shaw *et al. (123)* had observed a two to threefold increase in the blood lactic acid of ketotic cows after the oral administration of 6 lb. of glucose.

Schultz and Smith *(95)* and Reid *(78)* observed increased blood glucose levels in sheep and goats after the injection of propionate. Acetate produced no change in blood glucose, but butyrate had some depressing effect on blood glucose. It has been reported that the concentration of volatile fatty acids in the rumen of cows with ketosis is low and that the relative proportion of acetic acid is high *(11, 38)*. Brown *(9, 10)* obtained similar results but observed that this could be duplicated by the withholding of feed for 15 hours. These findings were confirmed by Schultz *(93)*. Thus, since cows with ketosis are usually in a fasting state, it appeared likely that the rumen volatile fatty acid picture obtained on cows with ketosis was due to fasting. More recently, Shaw *et al. (110)* observed that the total rumen volatile acids, as well as the proportions of acetic, propionic, butyric, and higher acids of three cows with proved ketosis but good

appetites, were normal. No lactic acid was observed in the rumen fluid of these cows.

In studies with normal cows, Hueter *(40)* and Hueter and Shaw *(41)* observed that the oral administration of 1.5 to 3 lb. of lactic acid equivalent, in the form of sodium lactate, increased blood lactic acid from three to fourfold within 2 hours, the blood levels reaching a peak at 2 hours but remaining above normal for 8 hours. Observations were not made beyond 8 hours. Total rumen volatile acids more than doubled by 8 hours, the increase being due primarily to propionic acid. The rumen lactic acid decreased gradually over the entire period. Blood glucose increased by more than 20%, approaching a maximum at 8 hours. Calcium lactate administration did not result in the rapid and prolonged increases in blood lactic acid and glucose observed with sodium lactate. It is apparent, therefore, that an appreciable amount of orally administered sodium lactate diffuses into the blood and that a substantial amount is converted into propionic acid by rumen microorganisms. Thus, lactates, especially the more soluble forms, offer promise for the treatment of bovine ketosis by making both lactic acid and propionic acid available as glucose precursors over an appreciable period of time.

The reports of Pennington *(71)* and Pennington and Sutherland *(72)* that propionic acid is converted into succinate in the presence of malonate by rumen epithelial tissue, apparently by a direct pathway involving carbon dioxide fixation, suggests the probability that the absorption of propionate, like that of butyrate, is via metabolism in the rumen epithelium and that acetic acid may be the only one of the three major rumen acids that is absorbed as such in appreciable amounts. As was noted above, lactic acid also passes rather freely from the rumen into the blood circulation. Thus, studies of factors which affect the absorption of rumen acids must be, in part, a study of factors which affect the metabolism of rumen epithelium.

6. *Therapeutic effect of glucose, glucose precursors, and glucose-sparing substances.* Hypoglycemia was demonstrated by Hupka in 1928 *(43)* to be associated with ketosis, and, since that time, carbohydrate therapy in bovine ketosis has received wide acceptance *(88, 123, 130)*. Sampson *(86)* was so impressed with results obtained by the intravenous administration of glucose that he stated, "Carbohydrate therapy is so specific for acetonemia of cows that, unless the condition is complicated by milk fever or some other disease, there is little to be gained from any other treatment." However, it is becoming generally accepted that the percentage of "glucose-resistant" cases is relatively large *(33, 57)*. In studies at the University of Maryland, cows with primary ketosis received from five to 14 daily injections of glucose without complete recovery. In a study of 306 cases of ketosis, Gingras *(32)* reported that 40% of the cases did not respond to one injection of 500 ml. of a 50% glucose solution. Roberts and Dye *(79)* recognized that there are appreciable numbers of glucose-resistant cases and proposed the continuous intravenous infusion of glucose for the treatment of such cases. This technique might well be used in obtaining additional information as to whether cows with primary ketosis, particularly the glucose-resistant

cases, are able to utilize glucose normally. Of the five cases treated by Roberts and Dye, it appears that only one may have been glucose-resistant and the results of this case were not too clear-cut, so that it is not possible to answer the question relating to the glucose utilization from the results of this study.

The feeding of soluble carbohydrate has not been particularly helpful in the prevention of bovine ketosis *(26, 100)*. This is not surprising, since Schambye and Phillipson *(92)* have shown that little, if any, soluble carbohydrate is absorbed directly from the rumen, since the rumen microorganisms readily convert the soluble carbohydrates to volatile fatty acids. Shaw *et al. (123)* did find that the oral administration of glucose was beneficial for the treatment of mild cases of ketosis. However, appreciable amounts were required and there is no assurance that the beneficial effect was not due to the lactic, propionic, or acetic acids produced from the glucose by rumen bacteria. It was demonstrated by Shaw *(100)* that the intravenous injection of glucose into cows with ketosis maintained the blood glucose at normal or above for only 80 to 100 minutes, after which it fell below normal again. This was confirmed by Roberts and Dye *(79)* and Holmes *(39)*. Thus, it is to be expected that single injections of glucose will not always be effective. Indeed, the surprising thing is that so many ketotic cows do recover after a single injection of glucose.

Several substances, which may be considered as glucose precursors or glucose-sparing substances, have been used with varying degrees of success. The first report of the use of a glucose precursor, other than various forms of sugar, was that of Seekles *(96)*, who used ammonium lactate. Approximately 4 oz. was administered per os twice a day for five or more days. Seekles stated, "The chronic (alimentary) form of ketosis gives a more favorable response to the ammonium lactate therapy than the acute (hormonal) type."

Sodium acetate, which may be considered as a glucose-sparing substance *(18)*, was used by Miller and Allen *(64)*, who reported that of 32 cows with ketosis that received up to 1 lb. of acetate per os per day for several days, 12 recovered without further treatment. Shaw *et al. (115, 116)* administered 1 to 1.5 lb. of sodium acetate per os per day for from 3 to 10 days to 15 cows with ketosis. In most of the cases there was an appreciable increase in blood glucose and an initial decrease in blood acetone bodies. Two cows exhibited a rapid clinical response, whereas the remainder either improved slowly or showed no improvement. Some of these animals would probably have recovered had the treatment been continued, but the response was so slow in most cases that other treatments were used.

After the reports that propionate had a hyperglycemic effect upon sheep and goats *(78, 95)*, Schultz *(93)* presented data showing that propionate was effective in the treatment of ketosis. Nineteen cows with ketosis were treated by oral administration of $\frac{1}{8}$ to $\frac{1}{2}$ lb. of sodium propionate daily. All of the cows showed increases in milk production and improvement of appetite. At the end of 10 days or less of treatment, blood glucose values were normal in all cows and blood ketones had returned to normal in all but three cows. Shaw *et al. (115, 116)* confirmed the report by Schultz that propionate was beneficial for the treatment of

bovine ketosis. Six cows exhibited very good recovery within 8 to 10 days, whereas five cows exhibited an increase in blood glucose but a slower clinical response, so that additional therapy was used. It was proposed that glucose or small initial doses of ACTH or cortisone be used as the primary treatment when either propionate or acetate is used and that the latter two substances be used as supportive treatments. Whether ketosis can be prevented by the oral administration of propionate has not been clearly established. However, Schultz *(94)* reported some advantage for propionate-fed cows in terms of lower blood ketones, higher blood sugar, and higher milk production for the first 5 weeks postpartum. Some limitations of propionate are its frequent failure to produce a prompt recovery and the necessity of daily drenching (twice per day) even after the cow regains her appetite, since propionate is relatively unpalatable. Johnson *(47, 49)* has used tripropionin, glycerol, and propylene glycol, finding that propylene glycol was the most effective. This was confirmed by Mills *(65)*, 500 g. per day being an effective dose when administered per os for several days. Since it is unpalatable, it must be given by drench or stomach tube. Shaw *et al. (109, 110)* have studied the use of various forms of lactates. Calcium lactate was found to be effective and more palatable than ammonium lactate. A combination of calcium lactate and sodium lactate appeared to be the most palatable, most cows eating between 5 and 10% mixed in the feed. Of 16 cows with ketosis that received 2 lb. of calcium lactate by drench followed by 1 lb. per day for 7 to 8 days, 14 exhibited good recovery, the blood glucose increasing from an average of 29.4 to 37.8 mg. % within 24 hours and remaining above 40 mg. %.

As was to be expected, however, on the basis of the studies of Hueter and Shaw *(41)*, the more soluble forms were found to be more effective than calcium lactate *(108)*. Sodium lactate increased the blood glucose of cows with ketosis more rapidly than calcium lactate and usually produced a more rapid clinical response. The upper level which can be administered safely was found to be approximately 1 lb. twice per day. Larger doses tended to be too laxative. When as much as 3 lb. were given in a single drench, the urine ketones frequently decreased from a high level to normal within 24 to 48 hours, the blood glucose increasing from a low level to a level above normal; rapid recovery followed in cows in which a severe laxative effect was avoided.

It is evident that the cow with ketosis is unable to obtain a sufficient amount of glucose, and, since much of it must be provided by glycogenesis from exogenous and endogenous substances other than glucose and the causative factor(s) is not merely an insufficient caloric intake, an alteration must take place, either in the exogenous precursors being provided or in glucogenesis from endogenous sources.

Although providing exogenous glucose precursors, such as propionate and lactate, aids greatly in restoring the metabolism of the ketotic cow to normal, there is little scientific evidence at the present time to suggest that bovine ketosis is due to a deficiency in the production of one or more of the rumen acids. Although lactic acid is present in only trace amounts in the rumen under many normal conditions *(16)*, it is known to reach appreciable amounts from time to

time and may be of more importance than has been generally recognized. Lactic and propionic acids probably exert their major antiketogenic action by providing a source of new glucose. They are more effective than glucose per os since they are absorbed in substantial amounts, whereas glucose is almost completely broken down by rumen bacteria and, therefore, only a part of it (lactic acid and propionic acid) is available to the body as a source of glucose. It does appear possible, however, that cows which are not resistant to the etiologic factors responsible for bovine ketosis may be rendered even more susceptible by diets that tend to produce less glucose-forming substances, such as lactic acid and propionic acid, or less glucose-sparing substances, such as acetic acid. β-hydroxybutyric acid is known to be a glucose-sparing substance in rabbits under certain conditions *(17)*, but butyric acid, derived for the most part from rumen butyric acid in the normal ruminant, has been reported to both increase and decrease blood glucose *(95)*. However, the picture is not too clear and the fact that very little butyric acid, as such, gets into the blood of the normal ruminant suggests that this does not play a role in the development of bovine ketosis.

The proportion of volatile fatty acids in the rumen may be changed to some extent by alterations in the diet. This suggests the possibility that ketosis may eventually be controlled to some extent by such alterations. For example, it has been reported by several groups that high concentrate–low roughage diets resulted in a relative increase in propionic acid production *(11, 133, 136)*. As noted previously, others *(74, 75)* have shown an increase in rumen lactic acid following the feeding of glucose, flaked maize, and other grains. Still others *(23, 143)* have shown an increase in the butyric and higher acids following the feeding of high protein diets. The comprehensive studies of Doetsch *et al. (16)* and Sirotnak *et al. (125)* have shown how numerous carbohydrates, fatty acids, and some amino acids are broken down by rumen bacteria, thus offering some promise that when sufficient information is available it will be possible to predict what the particular end products will be under any given set of conditions. This poses a problem of the optimum amounts and proportion of the various end products of rumen microorganisms required for the well-being of the animal and/or for milk production or growth and fattening.

CONCEPT OF KETOSIS AS AN ENDOCRINE DISTURBANCE

1. *Biochemical, histopathological, hematological, and replacement therapy studies.* The beginning of our present day concept of the possible role of the adrenals in the etiology of ketosis was the report in 1947 by Shaw *(103)* that an extract of the adrenal cortex was effective in the treatment of ketosis in dairy cows. A histopathologic study was initiated in 1946 by Hatziolos and Shaw *(34, 114, 118, 124)*, which was directed at the adrenal and pituitary glands but included various other endocrine glands and organs of cows that were sacrificed after the development of ketosis. Fourteen cows with ketosis were sacrificed for this study. The adrenals were enlarged and flabby and exhibited marked fatty infiltration with partial degeneration of the cortex. The medulla of the adrenal was not affected. Other changes observed were regressive changes of the anterior

lobe of the pituitary gland, involution of the thymolymphatic system, acute involution of the pancreas, gastrointestinal inflammation, and ulcers, nephrosis, and fatty changes in the liver. The pituitary of one of the cows that had developed ketosis for several consecutive years after parturition showed a rupture in the wall of the residual lumen, which resulted in a rather large sac filled with colloidal fluid. The pituitary of another cow that had developed ketosis for several consecutive years showed a depression and partial atrophy in the anterior lobe, apparently caused by mechanical pressure.

Recognizing that various types of stress tend to produce cellular changes and, in particular, that the ketotic cow is usually on a low caloric intake, studies were also made on lactating cows on a low level of energy intake postpartum. However, the significant changes observed in the anterior lobe of the pituitary, many of the changes in the adrenal, and the degree of enlargement and flabbiness of the adrenal glands were not observed in the fasting animal *(124)*. It was possible to reproduce many of the histopathologic changes by complete starvation for 8 days. However, the degree of fasting observed in many of the cows with ketosis was insufficient to produce the histopathologic changes observed in the endocrine glands of these ketotic cows. A few photomicrographs of this work were presented in another review *(106)*. Realizing that the histopathologic findings on the 14 cows sacrificed in various stages of ketosis were similar to the tissue changes which Selye *(98)* described as being characteristic of the alarm reaction phase of the adaptation syndrome, and recalling the early success in the treatment of ketotic cows with extracts of the adrenal cortex, cortisone acetate was used in the early part of 1950 for the treatment of ketosis with remarkable success. As a result, it was proposed in 1950 by Hatziolos and Shaw *(34)* and Shaw *et al. (114)* that bovine ketosis is due to an adrenal insufficiency, possibly associated with the pituitary-adrenocortical system. Later, more extensive studies were reported on the effective treatment of bovine ketosis with cortisone acetate, and an initial report was made on the effectiveness of adrenocorticotropic hormone (ACTH) in the treatment of bovine ketosis *(113)*.

In view of the fact that it has been proposed that primary ketosis in cattle is a manifestation of the adaptation syndrome as described by Selye, it may be well to discuss this syndrome rather briefly. The body shows a rather nonspecific and consistent reaction to a whole variety of adverse stimuli. Adaptation has been divided into three stages. In the beginning the stress causes a state of shock. This activates the defense mechanism and the body attempts to adjust itself. This stage is referred to as counter shock. The two phases are combined under the term "alarm reaction." After the alarm reaction the animal is more resistant to the same stress. This period is called the stage of resistance. It has been proposed that, during this stage, one severe stress factor causes the body to concentrate its efforts and direct its forces against this one factor and thereby renders it less able to meet new stresses which may arise. If a second stress factor (noxious agent) is superimposed over the original agent, the body will be incapable of adequately mobilizing its forces against what is now more than one factor at a time, the stage of resistance will be lowered, and a state of exhaustion results.

Most cows are able to adapt themselves to the normal stresses of parturition and early lactation. On the other hand, cows that develop ketosis year after year regardless of environment may do so because they are unable to adapt themselves to these normal physiologic stresses. If it is true that primary ketosis is due to a temporary adrenal insufficiency, absolute or relative, caused by a temporary exhaustion of the pituitary as the result of stress, it would appear that most ketotic cases are caused by unknown stresses superimposed upon the normal stresses incident to parturition and early lactation. The relative insufficiency of the adrenals, which is presumed to develop, results in a failure of the adrenals to produce enough glucocorticoids, with the result that blood glucose and carbohydrate reserves decrease and other changes take place, resulting in the ketotic syndrome.

Carlström *(12)* confirmed the early report of Shaw *(103)* that adrenal cortical extracts were beneficial in the treatment of bovine ketosis. He further stated that adrenal cortical extracts and desoxycortisterone corrected the hypokaliemia, which he reported to exist in cows with ketosis. He proposed that bovine ketosis is due to a reduced production of ACTH as the result of a high production of prolactin.

2. *Blood leucocytes and blood and urine glucocorticoids.* The normal cow at parturition and immediately postpartum is subject to considerable stress, as shown by the fact that there is a marked decrease in blood eosinophiles during the parturient period *(24, 62, 105, 116, 117)*. In the Maryland studies *(116)* a marked decrease was usually observed on the day of parturition and the low level was sometimes maintained for a few days followed by an increase. After the first few days postpartum there was a wide variation in the eosinophile levels. Several cows exhibited a low level of circulating eosinophiles for several days to several weeks postpartum. Ferguson *et al. (24)* reported that the total number of leucocytes decreased significantly immediately after parturition and later increased to about the precalving level. No change was observed in the percentages of either the neutrophiles or the lymphocytes, but the percentage of monocytes increased considerably. Merrill and Smith *(62)*, on the other hand, observed a decrease in the per cent of circulating lymphocytes and an increase in the neutrophiles.

Carlström *(12)* observed in cows with ketosis an increase in number of eosinophile leucocytes of 15 to 40% and an increase up to 60 to 80% in the number of lymphocytes, whereas the number of neutrophile leucocytes was low. It was noted by Shaw *et al. (116)* that the eosinophiles of ketotic cows vary from normal to high levels. These observations are in accord with the concept that bovine ketosis is due to a partial or relative insufficiency of the adrenals. In a continuation of these studies, B. C. Hatziolos in the Maryland laboratory has observed that cows with ketosis may be divided into two groups, one with relatively low eosinophiles and the other with normal to high eosinophiles. Recently, G. S. Bajwa in the same laboratory has completed studies on the circulating eosinophiles of cows with ketosis in which he has observed both low and normal eosinophiles. Where low eosinophiles were observed, there was a high percentage of

small lymphocytes, a low level of segmented neutrophiles, and the appearance of appreciable numbers of immature neutrophiles in the form of myelocytes, juveniles, and stabs. No change from normal was observed in the circulating monocytes. When the circulating eosinophiles were normal to high, the other leucocytes were more nearly normal.

Whereas the cows with ketosis in the Maryland area were about equally divided between low and normal to high eosinophiles, a much larger percentage of the cows in the Los Angeles area exhibited low eosinophiles. If this can be taken as evidence of an increased secretion of glucocorticoids, it might be postulated that these cows represent a more severe relative adrenal insufficiency, which could explain the fact that these cows usually require much larger doses of glucocorticoids than have been observed in other areas. In view of the hypothyroid theory of Robertson *et al. (81)* it is interesting to note that many of these cows are imported from iodine-deficient areas of the Northwest. It will not be surprising if it is eventually found that cows with ketosis may also be divided into two groups with respect to blood glucocorticoids.

Of particular interest is the report of Carlström *(12)* that in parturient paresis the neutrophile leucocyte count is high, often between 50 and 70%, and the number of eosinophile leucocytes and lymphocytes is low, which he noted would indicate increased function of the adrenal cortex. Similar observations were made by Garm *(29)*, Smith and Merrill *(128)*, and in studies at Maryland *(106)*. In the latter studies numerous cows had histories of parturient paresis followed by ketosis from 1 to 3 weeks later, for three or more consecutive years, suggesting a possible relationship in the etiology of these two disturbances.

If a temporary depression of the secretion of ACTH exists in cows with ketosis, such animals should not exhibit a decrease in blood eosinophiles following the injection of epinephrin. Such was shown to be the case by Shaw *(105)* and Shaw *et al. (116)*. Thirteen normal cows exhibited marked eosinophile decreases of 21 to 46% four hours after the injection of 5 ml. of epinephrin (1-1000), whereas of seven cows with ketosis, six exhibited no significant decrease in eosinophiles and one decreased 9%. Later studies on six normal cows maintained on a low energy intake demonstrated that the normal eosinophile response was diminished: Thus, it is apparent that the pituitary of the ketotic cow does not function normally, but it is not certain to what extent this may be due to lowered food intake. It should also be recognized that the epinephrin test has not proven to be too dependable in screening adrenal cortical insufficiency.

Puntriano *(76)* presented some interesting evidence in support of the concept that bovine ketosis is a pituitary-adrenocortical disturbance. Based on urine glucorticoid assays, a decrease in corticoadrenal activity was observed in two ketotic cows as contrasted with two normal cows. However, no work was done on fasted cows. Since cows with ketosis are usually in a fasting state, the use of fasted cows as controls would appear to be essential.

Vigue *(139)* reported a failure of cows to develop ketosis following administration of 2 g. of cortisone or 600 AVU of ACTH for as long as 2 weeks. In the Maryland laboratories, A. C. Chung has injected larger doses of both glucocorti-

coids and ACTH over a longer period of time without observing a hypoglycemia or ketonemia following cessation of treatment. These injections were made immediately prepartum and discontinued at parturition since cows are most susceptible to ketosis in the early postpartal period. It is apparent that it will be necessary to resort to more drastic measures to establish the role of the adrenals in the bovine.

A somewhat puzzling observation by Shaw *et al. (115, 116)* was the gradual decrease of blood eosinophiles to very low levels during the treatment of several cases of ketosis with propionate, followed by a rapid increase to normal levels as recovery took place. It appeared that propionate may have been acting as a stimulant to glucocorticoid secretion, directly or indirectly, and may explain in part why relatively small amounts of propionate are beneficial in the treatment of bovine ketosis since marked reductions in blood eosinophiles were usually observed within 12 to 24 hours following the treatment of ketotic cows with cortisone or ACTH.

It was reported by Robertson *et al. (81)* that the plasma 17-hydroxy-corticosteroid values of cows with ketosis were significantly higher than for normal cows, whereas the protein-bound iodine values were lower for cows with ketosis than for normal cows. They suggested that ketosis may be caused by a relative adrenal insufficiency, induced by low thyroid activity, the elevated blood corticoid values representing a failure of utilization or metabolism of the corticoids. This represents the first critical evidence that the adrenal insufficiency of bovine ketosis, assuming that our hypothesis is correct, is not mediated through the pituitary but may be mediated through the thyroid. For further information on the relationship of the thyroid to the adrenals the reader should consult the recent excellent review by Money *(66)*. These findings may prove to have great significance in elucidating the possible role of the adrenals in the etiology of bovine ketosis. The report is of interest in connection with the report by Reece *(77)* that thyroprotein is helpful in the treatment of ketosis. The data are somewhat difficult to appraise, however, in the absence of data on fasted controls or ketotic cows with good appetites. In the Maryland laboratory B. C. Hatziolos has observed a regression of the thyroids of cows with ketosis. This suggests that the low plasma protein-bound iodine values observed by Robertson *et al.* are due to the observed increase in glucocorticoids rather than to a basic hypothyroid state, since hypertrophy of the thyroid would be expected in the latter case. Likewise, there are no reports of an association of ketosis in the dam to goiter in the young calf, such as might be expected if a hypothyroid condition is the cause of bovine ketosis. The increase in plasma glucocorticoids appears to be significant and may require that we alter some of our concepts of the cause of the apparent adrenal insufficiency in bovine ketosis. It is true that cows with ketosis do not show as great a blood glucose response to ACTH, hydrocortisone, or some of the newer and highly potent glucocorticoids as normal cows, which may suggest a relative adrenal insufficiency. With the exception of cortisone acetate *(113)* more of these substances are required for treatment of cows with ketosis

than would be indicated on the basis of the physiologic response obtained on normal cows *(110, 111, 112, 113)*.

3. *Glucocorticoid and ACTH therapy.* The use of cortisone for the treatment of bovine ketosis was first reported by Hatziolos and Shaw *(34)* and Shaw *et al. (114)* in 1950. The first case to be treated received 900 mg. of cortisone acetate intramuscularly. Within 20 hours the cow regained her appetite, all signs of paresis disappeared, and the blood glucose rose from a low level to a normal level within 48 hours. Additional studies were reported *(113)* in which 14 cows with primary ketosis exhibited remarkable recovery following cortisone acetate therapy. The blood glucose response to an initial dose of 1.5 g. was much more marked than that obtained with 0.9 g. In subsequent studies this proved to be the typical pattern *(115, 116)*. Seventy-five cows with ketosis were treated with varying doses of cortisone. In most cases a suspension of cortisone acetate was injected intramuscularly. It was noted that doses of less than 1 g. given repeatedly were usually less effective than one dose of 1.5 g. The usual pattern observed was for the blood glucose to return to normal within 8 to 10 hours and to rise above normal within 24 hours, remaining above normal for two or more days before leveling off. The blood ketones or acetone bodies usually returned to normal by the third to fifth day. Blood eosinophiles decreased to low levels within 12 hours. Milk production usually showed the greatest improvement after 2 to 3 days. The appetite usually improved within 18 to 24 hours. Most cases were cured with one injection of 1.5 g. Larger doses produced an even more rapid and certain cure. For example, a rapid recovery was observed in one animal which received an initial injection of 1.5 g. of cortisone acetate followed by an additional gram 7 hours later. This cow again developed ketosis the following year and was given repeated injections of 500 to 750 mg. of cortisone acetate. Temporary responses followed by relapses were observed, and several weeks elapsed before the animal recovered, at the end of which time the cow was exhibiting marked emaciation and had developed albuminuria.

Dye *et al. (21)* treated 13 cases of bovine ketosis with 500 mg. of cortisone. Six recovered promptly without additional dosage. Two received additional injections of 250 mg., which the authors state was probably not needed. The remaining five required additional dosage, although three appeared to have complications. Much larger doses than this were shown to be required in the Los Angeles area by Shaw *et al. (111)*.

Although relapses have been observed from time to time, an initial response to a 1.5 g. dose of cortisone acetate has always been observed. It appears that cows which do not exhibit a good response either do not have primary ketosis or have primary ketosis complicated by other abnormalities. A temporary response may be expected in most cases, even when complications are apparent, if the condition is one of primary ketosis.

Intravenous injections of a ketotic cow with 200 and 300 mg. of cortisone alcohol, two days apart, resulted in a temporary improvement of symptoms and some increase in blood glucose but the improvement was followed by a relapse *(116)*. In these studies it was noted that cortisone acetate often increases the

blood glucose of ketotic cows to almost twice the normal values. Such increases were not observed in normal cows when similar dosages of cortisone acetate were employed. In these studies it was observed that the fasted cow exhibited some additional increase in blood glucose following cortisone administration, but not of the same order as that of the ketotic cow. Thus, it appears that the ketotic cow is far more sensitive to cortisone than is the normal cow, owing possibly to a hormonal imbalance in the ketotic cow.

Hydrocortisone acetate was first reported to be an effective treatment for ketosis by Shaw *et al. (115, 116)*. Four cows with ketosis responded well to intramuscular injections of 300 to 600 mg. of hydrocortisone acetate. In subsequent work at Maryland, however, hydrocortisone acetate has been less effective *(30)*. Of 13 cases of primary ketosis treated with 600 to 1000 mg. of hydrocortisone acetate, seven recovered without additional treatment, whereas six required additional treatment. It was observed that the blood glucose response of ketotic cows following the intramuscular injection of either 1 or 1.5 g. of hydrocortisone acetate was usually less than that observed from a similar dose of cortisone acetate. Hydrocortisone alcohol, on the other hand, is more potent than cortisone acetate. Of 32 cases of primary ketosis treated with intramuscular injections of 1 g. of hydrocortisone alcohol, 22 recovered without further treatment, seven required one additional treatment, and three required two additional treatments. The increase in blood glucose observed within 24 hours was greater than that usually observed with 1.5 g. of cortisone acetate. The differences are probably due to the fact that hydrocortisone alcohol is more soluble in blood plasma than either cortisone acetate or hydrocortisone acetate.

A combination of the quick-acting hydrocortisone alcohol and the slower acting hydrocortisone acetate was used in an attempt to increase the effectiveness of hydrocortisone. Nine cows with primary ketosis that received an intramuscular injection of 0.75 to 1 g. of a combination of the alcohol and acetate form of hydrocortisone (1:1) all recovered rapidly without further treatment.

The use of adrenocorticotropic hormone (ACTH) for the treatment of bovine ketosis was first reported by Shaw *et al. (113)* and was important in showing that the adrenal glands of the ketotic cow are still functional. The initial response of two cows treated with ACTH was good, but recovery was slow, probably due to the fact that an aqueous solution of ACTH was used, which would be expected to be dissipated rapidly. In subsequent studies *(115, 116)* on 37 cows with ketosis, which were treated with various forms of ACTH, it was observed that ACTH in gelatine was highly effective. These observations were confirmed by McAuliff *et al. (59)*.

The minimum effective dose of ACTH which would be applicable to most cases of primary ketosis is difficult to establish with present known limitations of determining the exact degree of deranged metabolism. While it is known that the adrenals undergo changes due to stress, which appears to be the major causative factor in bovine ketosis, these glands are still partially functional, since a dramatic response is obtained from ACTH. However, ACTH usually has a more marked hyperglycemic effect on normal cows than on ketotic cows *(116)*. This

provides further evidence that the adrenals of ketotic cows are not as functional as those of normal cows. Also, ACTH injections (100-300 I.U.) resulted in a marked reduction in the milk production of normal cows within 24 hours, followed by a return to normal within four days.

The question was raised as to why a relatively small dose of ACTH (100 I.U.) produces such a marked hyperglycemia in normal cows whereas 1.5 to 3.0 g. of cortisone acetate results in only a slight increase. Recent studies by Shaw *et al. (112)* demonstrated that intramuscular injections of normal cows with 5 g. of hydrocortisone alcohol produced a marked hyperglycemia and a sharp reduction in milk production, thus suggesting that the effect of ACTH on normal cows is merely that of eliciting a substantial increase in the production of highly active glucocorticoids by the adrenals. In studies at Maryland it has not been possible to confirm the report by Vanderplasschе *(138)* that desoxycorticosterone is helpful in the treatment of bovine ketosis.

It has been reported that the intramuscular injection of 1 to 3 g. of 11-ketoprogesterone is an effective treatment for bovine ketosis *(31)*. In studies at Maryland *(112, 129)* of 26 cows treated with 1.5 to 5 g. of 11-ketoprogesterone, 12 exhibited complete recovery with one treatment, and 14 required additional treatment. Blood glucose increased slowly, reaching a maximum at 48 hours. The clinical response was usually slower than that obtained with cortisone or hydrocortisone.

In studies conducted in the Los Angeles area *(111)* it was observed that larger doses of ACTH, hydrocortisone or a combination of hydrocortisone and hydrocortisone acetate were needed than were usually required in the Maryland area *(30)*. It was noted that when 1.5 to 2.5 g. of hydrocortisone were administered over a 2- or 3-day period, even the most difficult cases recovered quickly. Glucose injected intravenously was not helpful as a supportive for glucocorticoids and did not decrease the dosage of glucocorticoids needed for good recovery. The combination of hydrocortisone and hydrocortisone acetate (1:1) had about the same effectiveness as hydrocortisone alone. These studies have established a base or optimum dose by means of which other glucocorticoids may be evaluated.

It has been found that some of the new glucocorticoids have a great deal more potency for cattle than any of the above *(110)*. On the basis of the effective dosage for treatment of ketosis and effect on blood glucose and eosinophile levels of normal cows, metacortandracin was three to five times as potent as hydrocortisone, whereas 9-alpha-fluorohydrocortisone was observed to be between 10 and 20 times as potent as hydrocortisone. The effective dose of metacortandracin in the Maryland area was observed to be between 300 and 500 mg., with the upper level being preferred. For the 9-alpha-fluorohydrocortisone, 100 mg. appeared to be the optimum dose. Ten cows with ketosis all recovered rapidly following the injection of 100 mg., the average blood glucose increasing from 31.8 to 77.4 mg. % within 24 hours and still being at a high normal level of 46.3 mg. % between 4 and 6 days post-treatment. The urine ketones cleared up within 4 to 6 days. It is considered significant that, in the various studies on ACTH and glucocorti-

coids, the blood glucose response on normal cows was much greater than for ketotic cows, thus suggesting that an adrenocortical insufficiency, which the author believes to be involved in the etiology of bovine ketosis, need only be a relative insufficiency. The one exception is cortisone acetate in which the blood glucose response is greater for the ketotic cow at the lower dosages.

During the summer of 1955 additional studies on several of the new glucocorticoids were made in the Los Angeles area *(108)*. More of the glucocorticoids was usually required, as compared to the Maryland area, in confirmation of work done in the same area the previous year *(111)*. Two injections of 500 mg. each of metacortandracin for two consecutive days were required to get good results, whereas a single injection of 500 mg. was highly effective in the Maryland area. Two injections of 50 mg. each of 9-alpha-fluorohydrocortisone acetate were very effective. Repeated injections of ketotic cows in the Los Angeles area with this corticoid resulted in death, apparently from hypokaliemia, the animals exhibiting extreme flaccidity. It was noted that 100 mg. of this corticoid depressed plasma potassium as much as 50% within 24 hours. The use of potassium is indicated when this corticoid is used and perhaps with all corticoids and ACTH, since it was noted that hydrocortisone also depressed the plasma potassium of cows. In fact, it may be found advisable to administer potassium as an adjunct to all treatments.

Delta-1-dehydro-9-alpha-fluorohydrocortisone was about twice as potent and 9-alpha-chlorohydrocortisone about one-half as potent as 9-alpha-fluorohydrocortisone for the treatment of cows with ketosis.

Studies on normal cows demonstrated that all glucocorticoids have some depressing effect on milk production of normal cows, the depression reflecting their potency in increasing blood glucose and depressing blood eosinophiles. However, at the recommended optimum dosage for treatment (1500 to 2500 mg. cortisone, 1500 to 2000 mg. hydrocortisone, 600 to 900 A.V.U. ACTH, 500 mg. metacortandracin, 50 to 100 mg. 9-alpha-fluorohydrocortisone, 25 to 50 mg. delta-1-dehydro-9-alpha-fluorohydrocortisone, and 100 to 200 mg. 9-alpha-chlorohydrocortisone), the depressing effect on the milk production of cows with ketosis is negligible and is usually masked by the increase in milk production which occurs with the recovery of the animal.

In summary, it may be stated that glucocorticoid therapy has an advantage over all others because of ease of administration and rapid physiological effect. With the development of highly potent glucocorticoids, the use of these substances should soon be relatively inexpensive and will probably replace intravenous glucose therapy for the most part. For oral administration sodium propionate, propylene glycol, and calcium lactate, or a combination of more soluble lactates are the most valuable, especially when used as supportive treatments. Potassium administration may prove to be extremely valuable, especially when given with glucocorticoids or ACTH.

4. *Other nutritional aspects.* Minerals, vitamins, fat, and protein are grouped together in one section because they have not been proved to be associated with the development of bovine ketosis, although numerous reports have appeared in

the literature suggesting that ketosis is due to a deficiency of cobalt, vitamin A, thiamin, etc.

In a study by Duncan *et al. (19)* values obtained for plasma calcium, magnesium and chloride did not indicate a disturbance in the metabolism of these constituents. In ketosis the inorganic phosphorus values were somewhat low but were believed to be associated with the poor nutritional condition of the cows. Henderson *(37)*, Gingras *(32)*, White *(141)*, and others have suggested that cobalt was effective in the treatment of ketosis. The data are not very convincing, although it is possible that a secondary or fasting ketosis may exist from time to time in cobalt-deficient cows. Saarinen and Shaw *(83)* reported that there were no significant alterations in plasma potassium, inorganic phosphorus, or blood chlorides in bovine ketosis. Carlström *(12)*, on the other hand, reported potassium levels somewhat below normal and stated that potassium chloride administered intravenously was beneficial for the treatment of bovine ketosis.

In studies in the Los Angeles area in the summer of 1955, Shaw and Chung *(108)* observed what appear to be relatively low levels of plasma potassium in cows with ketosis, the average plasma potassium of 43 cows with ketosis being 16.2 mg. %. These values are in the range reported by Carlström but lower than those observed by Saarinen and Shaw *(83)* in the Maryland area. The low potassium values observed in the Los Angeles area are of interest in view of the observations made in 1954 *(111)* and confirmed in 1955 *(108)* that the per cent of cows in the Los Angeles area that are refractory to treatment is greater than for the Maryland area. However, before these data can be evaluated properly, it will be necessary to obtain data on plasma potassium of normal cows in the early postparturient period.

Since ACTH produces hypokaliemia in man and in the rat, and adrenalectomy increases serum potassium *(97)*, a low potassium might be considered as evidence against the hypothesis that bovine ketosis is due to an adrenal insufficiency. However, it is unsafe to generalize, since species differences may exist. Carlström *(12)* did report an increased serum potassium in cows with ketosis after the administration of adrenal cortical extracts and suggested that this indicated an adrenal insufficiency. It is possible that bovine ketosis may be a matter of relative adrenal insufficiency in which there is an actual increase in adrenal activity *(81)*, which would be responsible for the hypokaliemia. Thus, the apparent hypokaliemia observed may tend to support the conclusion of Robertson *et al. (81)* that there is an actual increase in blood glucocorticoids in cows with ketosis and that there is a relative, rather than absolute, adrenal insufficiency in bovine ketosis.

Breirem *et al. (6, 7)* in rather extensive studies in Norway were unable to prevent ketosis with supplements of phosphorus, copper, or cobalt and believed the ketosis associated with a cobalt deficiency was a fasting ketosis. They believed that an energy deficit played an important role in the high incidence of ketosis observed in Norway during the war. An interesting observation was that, whereas several cases of ketosis occurred among their experimental

animals, "not a single case appeared among the cows which received the alkali-treated straw."

Talsma *(132)* published a rather comprehensive review of the literature on ketosis, including a series of studies in the Netherlands. He stated that both potassium chlorate and sodium chlorate were beneficial for the treatment of ketosis and more effective than potassium chloride and concluded that the curing effect was due to the chlorate ion. Talsma stated that Seekles differentiates between an alimentary form of ketosis and a hormonal form, "whereby the hypophysis-adrenal system is very important."

MacKay *(57)* and Patton *(68, 69)* reported that ketosis was due to a vitamin A deficiency and could be cured by vitamin A therapy. However, Shaw *et al. (121)* demonstrated that the blood plasma carotene and vitamin A values were normal in eight cases of uncomplicated bovine ketosis and that the administration of from one million to four million I.U. of vitamin A, for as long as 3 weeks, had no value in the treatment of ketosis. Hayden *et al. (35)* confirmed the latter observation. Later, Shaw *(104)* made a detailed study of 13 cows which were maintained for 4 months prepartum and 3 weeks postpartum on an inadequate carotene intake. It was shown that a vitamin A deficiency per se did not result in the development of primary ketosis, even in the cows that were fasted during the postpartal period.

Thiamin was reported to be helpful for the treatment of ketosis by Carlström *et al. (13)* and Compton *(14)*. However, Shaw *(101)* obtained negative results by the administration, orally and intravenously, of massive doses of thiamin, alone or in combination with riboflavin, pyridoxine, niacin, pantothenate, inositol, para-aminobenzoic acid, and biotin. No one has been able to show a relationship between the composition of the ration and the development of ketosis.

In studies with 36 normal cows, Shaw and Cairns *(107)* observed that a high fat–low protein ration for 16 weeks prepartum, followed by a restricted caloric intake of 50% for 3 weeks, did not produce clinical ketosis, though a marked hypoglycemia did develop. Cows on high protein levels and high and low sugar levels were also studied with similar results. In the experiments of Shaw and Leffel *(120)* and Leffel *(56)* the level of protein in the ration was found to have no significant effect on the level of blood glucose or ketone bodies of cows on either a limited or liberal energy intake immediately postpartum. In studies on approximately 150 cases of ketosis in the Los Angeles area during the summers of 1954 and 1955, it was observed that the incidence of ketosis was approximately 10%, although almost all of the cows in that area are maintained on relatively high protein rations consisting of excellent quality alfalfa hay and/or freshly cut alfalfa and liberal amounts of a concentrate containing 16% or more protein.

It must be concluded that there is little evidence that either a high or low protein ration is conducive to the development of ketosis. In fact, all attempts to produce primary ketosis by nutritional means have met with failure. It is entirely possible that the development of primary ketosis may eventually be found to be associated with either feeding or management, but little or no concrete evidence of such is available at the present time. In fact, the incidence of

ketosis is relatively high in many herds which, on the basis of our present knowledge, are being fed rations which are nutritionally adequate in every respect. Field observations of a possible relationship of nutrition to the etiology of primary ketosis must be treated with reserve since ketosis occurs in cattle on widely different diets. That numerous cases are of metabolic rather than nutritional origin is indicated by the fact that many cows have been observed to develop ketosis for as many as three to six consecutive years regardless of dietary or environmental changes.

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INHERITANCE OF BLACK HAIR PATTERNS IN CATTLE LACKING THE EXTENSION FACTOR FOR BLACK (E). II. BLACKISH PATTERN[1]

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The effect of internal environment on the expression of genes is ideally demonstrated by the reversal of dominance exhibited in traits influenced or modified by relative levels of the sex hormones. The concept of sex-influenced inheritance is but vaguely understood, although generally accepted. Fundamentally, the sex of the heterozygote determines which of two phenotypes is expressed. Conditions of sexual dimorphism exhibited in animals heterozygous for certain autosomal alleles have been reported for pattern baldness in man *(8)*, certain types of horn formation in sheep and cattle *(6, 14)*, and "mahogany" hair color in cattle *(5, 12)*. The object of the study herein reported was to test further this fundamental concept, as well as to add to the knowledge of this specific trait.

Historically, the mechanism of inheritance which "mahogany" presumably demonstrates has been designated as sex limited *(5, 13, 15)*, sex controlled *(11)*, sex modified *(11)*, and sex influenced *(10)*. The term "sex limited" is inappropriate because of its current use in reference to traits in which the variability or even the manifestation of the trait is limited to one sex. The term "sex controlled" seems to be less indicative than "sex influenced." The use of "sex influenced" rather than "sex modified" is preferred only because it appears to be pre-empted for the trait herein described.

Wentworth in 1916 *(12, 13)* described the pigmented hair color of Melrose Good Gift, herd sire in the Kansas Station herd, as dark mahogany. The progeny by this bull and those from the six different mating types gave evidence that a sex-influenced trait was involved. The use of the word "mahogany" to describe this color now appears unfortunate, although its use before Wentworth's papers goes back at least to 1906. The Herd Book of the Ayrshire Society of Great Britain and Ireland describes the color of Ayrshires as "red of any shade, brown, or these with white, mahogany and white, black and white, or white, each colour distinctly defined." The acceptance of the term is indicated by its use in standard textbooks of genetics and animal breeding. One standard dictionary defines "mahogany" as a reddish-brown color. Another defines it as a reddish-red yellow hue. Natural mahogany wood may vary from blond to deep red. Although hairs of the latter color have been found in Ayrshire cattle, this color seems more descriptively called cherry red. Therefore, Bogart and Ibsen's desig-

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nation *(3)* of this pigment pattern and its absence as much or little blackish rather than mahogany and nonmahogany is more appropriate but nevertheless not consistent with recommended gene nomenclature and symbolism *(1)*, in that different alphabetic letters are used to symbolize alleles.

Baldwin *et al. (2)* have further clarified the description of blackish. They found that several areas of the body were involved in different frequencies to give a great range in the expressivity of this trait; established the maximum frequency of blackish to occur at approximately 2 years of age in females and approximately 1 year in males; and calculated the frequency of the gene for blackish *(Bs)* in both Ayrshire and Jersey breeds.

In light of these considerations and others presented herein, adequate terminology is desired. That which seems most pertinent to fill the deficiencies will be used throughout this paper.

Because blackish pattern is descriptive, it is suggested as a more pertinent term than mahogany for this trait. The use of the symbol *Bp* (blackish pattern) logically follows.

Because of the sexual dimorphism in the heterozygous condition, the use of large and small case letters is confusing and somewhat impertinent. Therefore, it is suggested that a subscript be used to designate that allele (i.e., the gene for blackish pattern) which is dominant in males (Bp_M).

EXPERIMENTAL PROCEDURE

Observations were made on the same cattle for the occurrence and expression of blackish pattern as used in the previously reported paper concerning blackish *(2)*. Discrepancies between the numbers of cattle reported in these two papers are due to the limitation of observations in the present study to females over 2 years of age and to males over 1 year of age. Further limitations were imposed by eliminating the cattle not expressing blackish *(bsbs)* and also those lacking sufficient pigmentation, i.e., *lwlw*, to determine the expression of the trait involved.

In this study the frequency of the gene for blackish pattern was derived by the gene frequency method reported by Snyder and Yingling *(10)*. The sample studied was divided into two phenotypic groups: those with and those without blackish pattern. The frequency of the two homozygous genotypes for the population was estimated by deriving the percentage of females that were blackish pattern, i.e., $Bp_M\ Bp_M$, and of males that were nonpatterned blackish, i.e., *Bp Bp*. The frequencies of the alleles were obtained by extracting the square roots of the relative frequencies of the respective homozygotes. The sum of the frequencies of these two alleles would approximate unity if the postulated mechanism of inheritance is valid.

Letting the symbol p equal the true frequency of the gene Bp_M, and q equal the true frequency of *Bp*, $p + q = 1$. Also $p^2 + 2pq + q^2 = 1$. Then p_o and q_o become the observed frequencies of the respective alleles and p_o^2 equals the proportion of females that were blackish pattern, and q_o^2 equals the proportion of

males that were nonpatterned blackish. The significance of the difference between unity and the sum of $p_o + q_o$ obtained was tested by a method devised by Green *(4)*.

RESULTS AND DISCUSSION

As a result of this study a terminology and symbolism more adequate to describe the trait and more in keeping with modern usage has been suggested. Blackish pattern is descriptive of the phenotype exhibited by the gene Bp_M. The subscript of this symbol signifies that this gene is dominant in males. The phenotypes of various combinations of sex and genotype are shown in Table 1.

TABLE 1

The phenotypes conditioned by the three genotypes of the alleles Bp_M and Bp in the presence of the gene for blackish, Bs

	$Bp_M\ Bp_M$	$Bp_M\ Bp$	$Bp\ Bp$
Male	Blackish pattern	Blackish pattern	Nonpatterned blackish
Female	Blackish pattern	Nonpatterned blackish	Nonpatterned blackish

The allele of Bp_M, i.e., Bp, is dominant in the female. Use of subscript $_F$ would appear logical to indicate dominance of this gene in heterozygous females, but its use would be superfluous.

A preliminary study on the blackish pattern trait suggested that the typical expression could be illustrated by blackish hairs over an area extending concavely from the withers to the rump (Figure 1). The observations made in this study verified Ibsen's conclusion *(5)* that there was considerable variation from the typical pattern. Variations in the areas having blackish hairs are found. These range from a pattern with only a fringe of blackish hair on the ventral side of the body and the more limited area of the thighs, shoulders, and neck, to

FIG. 1. Typical expression of the Blackish pattern gene as exhibited by a Jersey bull.

a pattern extending to the dorsal part of the body. For the purposes of this study, these variations were included as one phenotype.

There were 51 Ayrshire females over 2 years of age showing blackish pattern, $Bp_M\ Bp_M$, which represents 19.54% of 261 blackish Ayrshire females with *Lw*. The comparable figure for nonpatterned blackish males is 26, representing 39.39% of 66 with blackish.

Therefore:

$$p_o^2 = 0.1954 \text{ and } q_o^2 = 0.3939$$
$$p_o = \sqrt{p_o^2} = 0.4420$$
$$q_o = \sqrt{q_o^2} = 0.6276$$

Then:

$$p_o + q_o = 1.0696$$

The difference between this sum and 1.0 was found to be not significant.

Using the method of maximum likelihood on these same data, Overton *(9)* obtained a value of 0.44105 for the frequency of Bp_M (p_o).

There were 52 Jersey females over 2 years of age showing blackish pattern, $Bp_M\ Bp_M$, which represents 29.71% of 175 blackish Jersey females. The comparable figure for nonpatterned blackish males is 23.33% of 60. Therefore:

$$p_o^2 = 0.2971 \text{ and } q_o^2 = 0.2333$$
$$p_o = \sqrt{p_o^2} = 0.5451$$
$$q_o = \sqrt{q_o^2} = 0.4830$$

Then:

$$p_o + q_o = 1.0281$$

The difference between this sum and 1.0 was found to be not significant.

The fact that the blackish pattern trait fits the requirement of sex-influenced inheritance, when all variations are included, suggests that the variations are caused by modifying factors.

A consideration of the literature and preliminary observations led to the prediction that a freemartin heterozygous for blackish pattern $Bp_M\ Bp$ would show the blackish pattern trait because of the masculinizing effect of her intersexuality. A pertinent case to test this hypothesis was found in a 3-month-old Ayrshire heifer. The sire of this calf was blackish but lacked blackish pattern and, therefore, had a genotype *Bs* ___ *Bp Bp*. The dam lacked blackish pattern but showed blackish in the head, *Bs* ___ *Bp* ___. The blackish pattern calf was accordingly expected to be a freemartin with the genotype *Bs* ___ Bp_M *Bp*. Inquiry disclosed that she was born twin to a bull. Closer examination of the genitalia confirmed the freemartin diagnosis. Typical characteristics indicating

this abnormality were elongated guard hairs extending from the vulva, enlarged clitoris, and an abnormally short vaginal canal with no cervical opening.

SUMMARY AND CONCLUSIONS

The term blackish pattern is suggested to replace mahogany.

The symbols Bp_M and Bp are suggested to represent the genes responsible for blackish pattern and absence of blackish pattern, respectively. The subscript $_M$ indicates the allele exhibiting dominance in males.

A gene frequency analysis of the data obtained in this study confirms that blackish pattern is a sex-influenced modifier of blackish. Blackish pattern appears to act as an extension factor by extending the blackish hairs over the body in a sexually dimorphic manner in the heterozygote.

The frequency of the gene for blackish pattern as determined from the samples studied is 0.44 for Ayrshires and 0.54 for Jerseys.

The freemartin thought to be heterozygous expressed the blackish pattern trait, presumably because of its intersexual condition.

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THE EFFECT OF LECITHIN, CHOLINE, AND METHIONINE ON THE VITAMIN A AND CAROTENE PLASMA LEVELS AND LIVER STORES OF YOUNG DAIRY CALVES[1]

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An efficient utilization of carotene and vitamin A is essential for the health of the young dairy calf, especially when the available intakes are equal to or below the actual needs of the calf. It is of fundamental importance, therefore, to determine if various methylating agents might conceivably aid more efficient utilization of carotene and vitamin A by the young dairy calf.

Conflicting data have been published concerning the effect of lecithin on the absorption and utilization of vitamin A in calves and rats. Esh *et al. (7, 8)* observed that when lecithin was fed along with vitamin A during the later stages of the gestation period a greater amount of vitamin A was transferred to the colostrum as well as stored in the fetal livers by way of the placenta. These same workers also found that calves fed a skimmilk ration supplemented with lecithin and vitamin A gained in body weight and showed blood plasma levels of vitamin A comparable to a colostrum-fed group of calves. Esh and Sutton *(6)* observed that the feeding of lecithin plus vitamin A or a carotene supplement stimulated greater weight gains and an increased liver shortage of vitamin A in laboratory rats than did vitamin A or carotene fed alone. Choline fed in addition to these supplements did not change the liver stores of vitamin A when compared to a group supplemented with vitamin A alone. Adlersberg and Sabotka *(1)* reported much higher vitamin A blood levels in man after the ingestion of soybean lecithin. Scharf and Slanetz *(12)* observed a marked increase in the liver stores and blood plasma levels of vitamin A in rats after feeding soybean lecithin but reported no increase or decrease of blood plasma levels or liver stores of vitamin A by the presence or absence of choline. Slanetz and Scharf *(13, 14)* later reported that the addition of soybean phosphatides to a ration supplemented with vitamin A or carotene produced better gains in rats. These same workers postulated that soybean lecithin contains an unknown factor essential for the utilization of vitamin A and carotene in the rat. Bently and Morgan *(2)* reported that rats fed a low fat, low protein ration supplemented with choline showed higher vitamin A stores in the liver than when choline was omitted from the ration. The addition of carotene to the same ration produced more vitamin A deposition in the liver, which was not affected by the presence or absence of

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choline. Popper and Chinn *(11)* observed in an experiment with rats that liver stores of vitamin A were dependent upon the intake of choline. A deficiency of choline produced fatty livers that were low in vitamin A. Thorbjarnarson and Drummond *(15)* reported that the addition of choline to a choline-deficient diet fed to rats brought about a decrease in fat and vitamin A content of the livers. Clayton and Baumann *(4)* observed that even during a choline deficiency vitamin A was found to be normally distributed in the livers of rats.

This experiment was conducted to study further the effect of three lipotrophic agents, lecithin, choline, and methionine on the absorption and utilization of vitamin A and carotene by young dairy calves.

EXPERIMENTAL PROCEDURE

Fifty-four 2-day-old Jersey male calves were used in a 42-day experiment which was conducted in three phases during the years 1950, 1951, and 1954. The calves were selected from the Kentucky Agricultural Experiment Station herd and two other privately owned herds located nearby. Two calves each year were randomly assigned to receive one of nine different treatments. The treatments were as follows:

Group 1—basal control ration
Group 2—basal plus 25,000 I.U. vitamin A
Group 3—basal plus 25,000 I.U. vitamin A, plus 3 g. DL-methionine
Group 4—basal plus 25,000 I.U. vitamin A, plus 2 g. choline chloride
Group 5—basal plus 25,000 I.U. vitamin A, plus 10 g. vegetable lecithin
Group 6—basal plus 25,000 I.U. carotene (beta)
Group 7—basal plus 25,000 I.U. carotene, plus 3 g. DL-methionine
Group 8—basal plus 25,000 I.U. carotene, plus 2 g. choline chloride
Group 9—basal plus 25,000 I.U. carotene, plus 10 g. vegetable lecithin

The amounts of the supplements shown were the amounts administered per calf daily. The vitamin A and carotene were administered orally in a gelatin capsule. DL-methionine and choline chloride were dissolved in water and added to the milk. The lecithin was formed into a ball and forced down the calf's throat just prior to milk feeding. All supplements were fed at the time of or just prior to the afternoon milk feeding.

The calves were allowed to remain with their dams until 2 days of age, at which time they were placed in individual calf pens and hand-fed whole milk at a rate of 10 lb. per 100 lb. of body weight. Supplemental feeding was started when the calves were 9 days of age and continued daily until the animals were slaughtered.

The basal grain mixture, which was low in carotene and vitamin A, was fed ad libitum beginning when the calves were 2 weeks of age. The grain mixture was composed of 12 parts ground white corn, 12 parts wheat bran, 24 parts dried skimmilk, and 1 part each of salt and steamed bone meal. Beet pulp constituted

the roughage portion of the ration, which was fed ad libitum and separate from the grain mixture.

Blood samples were drawn from the jugular vein when the calves were placed on experiment and at weekly intervals thereafter for vitamin A and carotene analysis. The blood was cooled immediately, and plasma vitamin A and carotene values were determined by the method of Kimble *(10)*. An Evelyn Photoelectric Colorimeter was standardized periodically by using crystalline beta carotene dissolved in petroleum ether and crystalline vitamin A acetate in cottonseed oil dissolved in chloroform.

The calves were slaughtered when 42 days of age, and the livers and kidneys were removed, frozen, and stored until analyzed. At the time of analysis the organs were sliced with a meat saw and ground in a food chopper while still frozen to prevent loss of moisture. The entire liver or kidney was mixed thoroughly, and aliquots were removed for analysis of dry matter, ether extract, vitamin A, and carotene. The total solids were determined by drying the samples either under vacuum over sulfuric acid or in a vacuum oven at 60° C. These dried samples were then extracted with anhydrous ethyl ether for approximately 30 hours to determine the per cent ether extract present. Vitamin A and carotene values were determined on the fresh livers and kidneys by the alkali digestion method outlined by Davies *(5)*.

RESULTS AND DISCUSSION

The summarized data showing the effects of the various supplements on the plasma levels of vitamin A, when vitamin A was fed, are presented in Table 1. The control calves, Group 1, exhibited much lower plasma levels of vitamin A than the supplemented calves. This is in agreement with Hibbs *(9)*, who found

TABLE 1

Mean vitamin A and carotene values of blood plasma and livers of calves supplemented with vitamin A plus lecithin, choline, and methionine

Group	Vitamin A /100 ml. plasma	Carotene /100 ml. plasma	Vitamin A /g of fresh liver	Carotene /g of fresh liver
1—Controls	9.6	18.0	12.6	0.5
2—Vitamin A alone	17.6	19.2	102.2	0.3
3—Vitamin A plus methionine	15.0	17.9	123.7	0.5
4—Vitamin A plus choline	16.1	18.0	132.9	0.2
5—Vitamin A plus lecithin	20.1	27.0	113.2	0.5

that the feeding of vitamin A alone in capsule would increase the plasma level of this vitamin. An analysis of variance was used to test for significant differences existing between Groups 2, 3, 4, and 5. These data along with the variance components are presented in Table 2. Group 5, receiving vitamin A plus lecithin, showed significantly higher plasma levels of vitamin A (5% level) than did Groups 3 and 4 receiving vitamin A plus methionine and vitamin A plus choline, respectively, but not significantly higher than Group 2, which was supplemented with vitamin A alone. Since Group 2 served as the control animals for the evalu-

TABLE 2

Analysis of variance
(Vitamin A plasma levels of calves fed vitamin A plus various supplements)

Source of variance	D.F.	Sum of squares	Mean square	F. ratio
Total	119	6020.89		
Age	4	104.95	26.2	
Treatments	3	427.55	142.5	3.68[a]
Years	2	1387.31	693.6	17.9[b]
Age × treatments	12	281.30	23.4	
Age × years	8	199.88	25.0	
Treatment × years	6	371.16	61.9	
Error	84	3248.74	38.7	

[a] Significant at the 5% level.
[b] Significant at the 1% level.
Treatments—Group 5 over 3 or 4 years; 3rd over 1st and 2nd; 2nd over 1st.

ation of the three lipotrophic agents, the differences obtained do not bear any significance. As can be observed from the variance components, there was considerable variation in response between years, which could probably be attributed to the better general health of the calves in the later years. These differences were significantly higher in favor of the last year (1954) as compared to the first and second year, and in the second year (1951) differences were significantly higher than in the first year (1950). In general the greatest response to the various supplements came within the second or third week after supplementation, followed by a downward trend.

Data concerning the plasma levels of carotene when vitamin A was fed are also presented in Table 1. An analysis of variance was used to test for significant differences as similarly outlined in Table 2 for the plasma vitamin A values. There were no significant differences between treatments; however, all groups of supplemented calves showed significantly higher plasma levels of carotene the first and second week after supplementation than was observed at the end of the experiment, or sixth week. These data follow a similar trend as that found for vitamin A, except that the decrease as the calves became older was not significant in the later case. It is of interest to note that the plasma levels of carotene were found to be significantly higher (5% level) the first year, which is the opposite of the plasma level of vitamin A. This would indicate greater conversion of the carotene to vitamin A in the later years. Liver vitamin A values of calves fed the various supplements plus vitamin A are presented in Table 1. There were no significant differences between any of the supplemented groups in the liver stores of vitamin A; however, liver stores in all supplemented groups were significantly higher than in the control calves, Group 1. There was a trend toward higher liver stores of vitamin A when the three lipotrophic agents were fed along with vitamin A. These data were not consistent enough to draw definite conclusions concerning the ability of these supplements to enhance the utilization of vitamin A as measured by liver stores.

These data support findings of Hibbs and Krauss *(9)* and Braun *(3)* that the plasma level of vitamin A is not a good criterion of the liver stores of this vitamin except when liver stores of vitamin A are low. Some calves were found

to exhibit high plasma levels of vitamin A and low liver stores of this vitamin. Calves on experiment during 1954, showed somewhat higher liver stores of vitamin A than the calves receiving the same treatment in the years 1950 and 1951. As was pointed out previously, these calves appeared to be in a better state of health, as reflected by better gains and fewer calf losses than observed in previous years of the study. When vitamin A was fed, the various supplements did not appear to affect the liver stores of carotene.

The effect of the various supplements on the plasma levels of carotene when carotene was fed is presented in Table 3. A statistical analysis of these data showed a significant increase at the 1% level of probability in the absorption of carotene, as measured by plasma levels of carotene, with the feeding of methionine over carotene alone (Group 6) and carotene plus lecithin (Group 9). A significant increase in plasma levels of carotene at the 5% level of probability was found in the group fed choline over Groups 6 and 9. The feeding of lecithin in addition to carotene appeared to have little effect on the plasma levels of carotene. A summary of the analysis along with the variance components is given in Table 4. All of the carotene-supplemented groups showed a significantly higher plasma level of carotene than the nonsupplemented controls (Group 1). This indicates that the basal ration alone was insufficient in carotene to maintain a normal level of plasma carotene. As was true with the vitamin-supplemented calves, the carotene-fed calves showed significantly higher plasma levels of carotene in the later years of the study.

TABLE 3

Mean carotene and vitamin A values of blood plasma and livers of calves supplemented with carotene plus lecithin, choline, and methionine

Group	Carotene /100 ml. plasma	Vitamin A /100 ml. plasma	Carotene /g of fresh liver	Vitamin A /g of fresh liver
1—Controls	18.0	9.6	0.5	12.6
6—Carotene alone	83.5	15.6	1.4	11.6
7—Carotene plus methionine	137.4	19.3	1.2	19.2
8—Carotene plus choline	124.0	18.2	1.4	10.3
9—Carotene plus lecithin	90.8	17.2	0.9	14.8

TABLE 4

Analysis of variance

(Carotene plasma levels of calves fed carotene plus the various supplements)

Source of variance	D.F.	Sum of squares	Mean square	F. ratio
Total	119	685,337.9		
Treatments	3	60,563.7	20,187.9	4.98[a]
Age	4	25,582.7	6,395.7	1.58
Years	2	147,233.2	73,616.6	18.17[a]
Age × treatments	12	27,040.7	2,253.4	0.56
Age × years	8	10,389.1	1,298.6	0.32
Years × treatments	6	74,261.7	12,376.9	3.05[a]
Error	84	340,266.8	4,050.8	

[a] Significant at 1% level.

Treatments—at 1% level, Group 7 over 6 and 9; at 5% level, Group 8 over 6 and 9.

Years—3rd year over 1st and 2nd (1% level); 2nd year over 1st (1% level).

As can be observed in Table 3, the various supplements had very little effect on the storage of carotene in the liver when compared with the group fed carotene alone; however, all supplemented groups showed higher liver carotene values than did the control calves. Here again, there appears to be very little correlation between the blood plasma level of carotene and the liver stores of carotene. Considerable variation was observed between calves within groups in this respect. Methionine was the only supplement that appeared to have any appreciable effect on the liver stores of vitamin A when carotene was fed. These data indicate that very little, if any, conversion of carotene to vitamin A occurred, except with the methionine-fed calves, when a vitamin A and carotene-deficient ration was supplemented with carotene. Contrary to these data, carotene supplementation increased the plasma levels of vitamin A, but none of the supplements appeared to have any addictive effect.

TABLE 5

The effect of the various supplements on the average daily gain, per cent dry matter,[a] and ether extract[a] in the liver and kidneys of the calves

Supplements	Av. daily gain	Liver		Kidneys[b]	
		Dry matter	Ether extract	Dry matter	Ether extract
	(lb.)	*(%)*	*(%)*	*(%)*	*(%)*
Group 1—Controls	0.68	27.9	1.97	21.3	1.91
Group 2—Vitamin A alone	0.62	28.3	1.93	20.9	1.81
Group 3—Vitamin A plus methionine	0.59	28.8	1.98	21.2	1.70
Group 4—Vitamin A plus choline	0.70	28.6	1.82	21.0	1.80
Group 5—Vitamin A plus lecithin	0.79	29.1	1.93	20.1	1.61
Group 6—Carotene alone	0.66	28.8	2.04	20.8	1.81
Group 7—Carotene plus methionine	0.48	29.1	2.11	20.3	1.73
Group 8—Carotene plus choline	0.57	28.9	2.30	20.9	1.71
Group 9—Carotene plus lecithin	0.65	28.9	1.97	21.4	2.03

[a] Fresh basis.
[b] Average of 4 calves used in 1950 and 1951.

As can be observed in Table 5, there were no great differences between any of the groups in respect to the per cent dry matter or ether extract in either the liver or the kidneys. The differences that did exist were not statistically significant, although it appears that the carotene supplementation had a tendency to increase the per cent fat in the livers.

The mean per cent fat in the liver and kidneys for each group is given in Table 5. Close observation of the livers revealed no noticeable defects or excessive storage of fat even when choline, methionine, and lecithin were not fed. Calves used in this experiment during 1954, regardless of ration, had less fat in their livers than the calves used during 1950 and 1951. This is difficult to explain, since the treatments were the same for all years; however, it might be partially due to the fact that during 1954 the calves made better gains and were less affected by scours and other complications.

The effect of the various supplements on the growth of the calves is summarized in Table 5. It must be pointed out that considerable variation existed between calves on the same treatment in respect to weight gains during any

particular year. This was probably due to the general health of the calves, since the basal ration was not formulated to produce fast growth but to be deficient in carotene and vitamin A. Even though only two calves were used per treatment each year of the study, all calves regardless of treatment tended to gain in weight faster with each succeeding year of the study. The average daily gains for all of the calves irrespective of treatment during 1950, 1951, and 1954 were 0.54, 0.61, and 0.80 lb., respectively. This was probably due to fewer cases of scours during the last 2 years. None of the supplements appeared to have any great effect on the weight gains of the calves, although the calves receiving lecithin with carotene or vitamin A tended to make slightly better gains than other supplemented groups.

SUMMARY

Fifty-four 2-day-old male Jersey calves were used to study the effect of three lipotrophic agents, DL-methionine, choline chloride, and vegetable lecithin, on the absorption and utilization of vitamin A and carotene. The feeding of these three agents plus vitamin A did not materially increase the plasma levels or liver stores of vitamin A above those of a group receiving vitamin A alone.

The addition of either choline or methionine plus carotene to the ration of calves caused a significant increase in the absorption of carotene as measured by plasma levels of carotene. All treated groups showed significantly higher plasma carotene levels than did the untreated controls. None of the lipotrophic agents had any significant effect on the liver stores of carotene when carotene was fed, but the feeding of carotene per se increased liver stores of carotene but not of vitamin A, although blood plasma values of vitamin A were increased. None of the supplements appeared to have any appreciable effect on the per cent ether extract or total solids in the livers and kidneys.

The absence of consistent results due to the addition of either methionine, choline, or lecithin to the supplementary vitamin A would indicate, under the conditions of this experiment, that they have little value in increasing the absorption or utilization of vitamin A in the young dairy calf. The results might have been more convincing if lower levels of the vitamin A or its precursor had been fed.

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OVARIAN FUNCTIONS, INTERVALS BETWEEN ESTRUS, AND CONCEPTION RATES IN DAIRY CATTLE[1]

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A considerable number of research reports giving the intervals between estrus are available. These also list the percentage of females that do not show estrus at the expected time, but the ovarian condition or the causes of these irregularities either were unknown or were not described.

This experiment was conducted to determine ovarian conditions or functions and their influence on regularity of estrus and conception rates in dairy cattle. Obviously, such knowledge would provide more complete information about reproduction in dairy cattle, especially on the advisability of recommending service to cows during irregular estrus as determined by the chances for conception.

Chapman and Casida *(6)* reported that 40% of 690 clinically normal cows showed estrous cycles less than or greater than 17 to 24 days. Asdell *et al. (2)* found that 16% of 167 estrous cycles did not fall within the period of 18 to 24 days. Ellenberger and Lohmann *(7)* reported that 30% of 274 estrous cycles observed in cows occurred at intervals less than or greater than 17 to 25 days. Among these, 8% occurred in less than 17 days and 22% after more than 25 days. Roark and Herman *(14)* found that 20% of 504 periods did not fall within the range of 18 to 24 days.

Moeller and VanDemark *(10)* accumulated data on 4,885 intervals between two consecutive artificial inseminations of cows and found that 44% of the total number of returns to service did not fall between the expected 18 to 25 days. However, only 4% of the returns fell between 2 and 17 days. Olds *(11)* reported that among 5,304 cows returning for second service in routine artificial breeding, 34.1% returned as late as 37 days or more. Olds *et al. (12)* reported that only about 40% of cows and heifers that failed to conceive returned in heat during the expected 18- to 24-day interval.

The consistent results in these reports show that irregularity of estrus in dairy cattle is an important problem to the breeder. Additional information about ovarian conditions and breeding efficiency associated with these irregular estrous periods may be very useful to keep cows at their maximum productive and reproductive levels.

Garm *(8)*, Wiltbank *et al. (18)*, and Casida and Chapman *(3)* stress the role that heritability plays for a predisposition to cystic ovaries, but it is not possible to consider heredity in this report. Asdell *(1)* prepared an excellent summary on the use of hormones in the treatment of sterility and the response that can be expected.

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Tanabe and Casida *(15)* reported on the nature of reproductive failures in cows listed as repeat breeders. Of 104 cows, apparently normal but with poor breeding records, half were slaughtered 3 days after service and 66% of these were found with fertilized ova. The other half slaughtered at the end of the 34th day had only 23% with embryos. This was a death rate of 65% and indicated that infections and other causes disturbed pregnancy in these selected cows with reproductive difficulties.

Salisbury *et al. (16)* reported that semen of different bulls showed significant differences in the incidence of embryonic mortality, and that these were negatively and highly significantly correlated with the original fertility level of the bulls. However, in addition to these factors associated with reproductive infections of the male or female, there are many irregular estrous periods not associated with embryonic mortality. It was the purpose of this research to investigate irregular estrous cycles in a herd with high levels of fertility under normal management conditions.

EXPERIMENTAL METHODS

Cows freshening at the main barn for the Cornell University dairy herd were assigned alternately, at random, to the experimental and control groups. All cows were used for the experiment, including 8.5% with retained placentae and 7.5% with metritis at calving in the experimental group. The control group had 7% of the cows with retained placentae and 5.5% with metritis after calving. The period covered by this research extended from 1948 to 1953, and each of the experimental and control groups comprised a total of 200 cows. These consisted of 127 Holsteins, 35 Brown Swiss, 20 Jerseys, and 18 Guernseys in each of the two groups.

To determine the relationship of the condition of the ovary to the regularity and frequency of estrus, the ovaries and reproductive organs in the experimental group were examined once weekly, or more frequently when desirable. At the time of the next expected estrous period the examinations were made at daily intervals or even more frequently if necessary, especially during silent estrous periods. The development and retrogression of the corpus luteum and other ovarian changes, such as follicle development, were carefully noted and recorded.

The ovarian examinations to determine changes were the only differences between the experimental and control groups. Both groups received recommended veterinary treatment for reproductive difficulties, especially for cystic ovaries (atretic large follicles) when these were indicated by too frequent estrus. Treatments for complete absence of estrus were provided only after long intervals of time, and none were provided before 90 days postpartum.

The cows were turned out into the exercise lot twice daily and were carefully observed for heat during a period of 15 to 30 minutes. In addition, some systematic checks were made with "indicator" females or "teaser" bulls, especially during silent heat periods. "Mounting" and "standing" were taken as indication of estrus. Heat expectancy lists were used as an aid to detect cows in estrus.

RESULTS AND DISCUSSION

The average number of days from parturition to first estrus for the experimental group was 43.7 as actually determined from the ovaries, but 49.4 from the apparent or external indications of estrus. The average for the control group was 50.9 days. The distribution of the first estrus after calving is summarized in Table 1. Results from the ovarian examinations showed 81.5% of the 200

TABLE 1

Actual and apparent distribution of the postpartum estrus

	Experimental group				Control apparent		Total apparent	
	Actual[a]		Apparent[b]					
	(No.)	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(No.)*	*(%)*
First 30 days	60	30.0	50	25.0	53	26.5	103	25.75
31 to 60 days	103	51.5	91	45.5	81	40.5	172	43.00
61 to 90 days	31	15.5	46	23.0	52	26.0	98	24.50
Over 90 days	6	3.0	13	6.5	14	7.0	27	6.75
Total	200	100.0	200	100.0	200	100.0	400	100.00

[a] Actual from ovary examination at regular intervals.
[b] Apparent from external indications or expressions of estrus.

experimental cows had the first postpartum estrus during the first 60 days after calving. An interval of 90 days included 97% of the 200 experimental cows. For this group, actual heat from external indications or expressions of estrus was demonstrated by 70.5 and 93.5%, respectively, during the intervals of 60 and 90 days after calving. However, 35 of these cows had 49 silent (unobserved or quiet) estrous periods before the first externally indicated estrus.

In the control group 67.0% of the cows showed estrus before 60 days or less after parturition, and 93.0% by 90 days postpartum.

After the first estrus following calving had been observed, the intervals between estrus and regularity of estrus were studied. A total of 28 cows (14%) in the experimental group and 21 cows (10.5%) in the control group conceived on the first service during the first estrus after parturition. Obviously, information on these cows from one estrus to the next was not available unless they had a silent estrus previously, which included 20 of the cows in the experimental group.

The number and percentage of normal and abnormal intervals between estrus are presented in Table 2 for the 200 cows in the experimental group. Some of the cows appear in several of the listings, and therefore the totals are not included in the column for number or per cent of cows. Under the column headed "estrus," the percentage of estrus with cystic ovaries is not listed, since the frequently recurring estrus for this abnormal condition would have too great an impact on the figures in this column. The 20 cows with 84 estrous periods due to cystic ovaries would overshadow the other normal or abnormal estrous periods. Casida and Chapman *(3)* reported that 7% of the cycles showed the occurrence of cystic ovaries. However, the irregular recurring estrus from cystic ovaries often does not leave a clearly defined cycle.

TABLE 2

Number and percentage of normal and abnormal intervals between estrus as determined from ovarian examinations for the 200 cows in the experimental group[a]

	Cows		Estrus interval	
	(No.)	*(%)*	*(No.)*	*(%)*
Cystic ovaries	20	10.0	84	[b]
Normal estrous periods (18-25 days)	141	70.5	302	60.4
Estrous period interval less than 18 days—not cystic	7	3.5	7	1.4
False estrus (less than 18 days) [c]	4	2.0	4	0.8
Estrous period interval over 25 days [d]	(108)	(54.0)	(138)	(27.6)
Silent estrus previous to first postpartum heat	35	17.5	49	9.8
Silent estrus after the first postpartum heat	33	16.5	44	8.8
Persistent corpus luteum	60	30.0	76	15.2
Smooth, nonfunctional ovaries	15	7.5	18	3.6
Total	—	—	500	100.0

[a] Among the 200 cows in the group 28, or 14%, conceived on the first service during the first heat after parturition. However, 20 of these cows had a previous silent estrus. The results in this table are based on normal herd-management procedures for which cows are listed for service 60 to 90 days after the previous calving. In view of this, the percentage of cows under each condition is based on the total 200.

[b] Percentage of estrus for cystic ovaries is not included. The frequency of estrus from cystic ovaries overshadowed the other normal and abnormal estrous periods, owing to the frequently recurring estrus. The percentages in the last column are based on actual intervals between estrus, as determined by ovary examinations rather than on total number of estrous periods. If number of estrus instead of intervals of estrus were considered, then the estrus at which conception occurred would have to be included in total numbers for calculation of percentages.

[c] False estrus describes the condition when estrus was indicated but no follicle was present on the ovary and/or estrus was not followed by the development of a corpus luteum.

[d] Placed in parentheses since these are included in the last 3 groups listed. Occasionally the same cow appeared in several of the groups as reflected in the total numbers for the column "cows," and therefore totals are not listed in the first 2 columns.

As listed in Table 2, 141 cows (70.5%) in the experimental group had 302 normal estrous periods between 18 and 25 days. They comprised 60.4% of the total number of 500 estrous periods. Exclusive of cystic ovaries, only 2.2% of the estrous periods were less than 18 days, including 0.8% which were false estrus, all with an interval of less than 18 days. The 138 (27.6%) estrous cycles with an interval over 25 days were due to silent estrus, persistent corpus luteum, and smooth, nonfunctional ovaries. The percentages of each were 8.8, 15.2, and 3.6, respectively. In addition to the 8.8% of silent estrual periods after the first postpartum heat, there were 9.8% before a first indicated estrus after calving, to bring the total of silent estrus to 18.6%. This is lower than 27.3% reported by Kidder *et al. (9)*. Cows with silent periods were often tested with cows and bulls and tried for natural breeding when the follicle indicated estrus, but they would not stand when mounted.

In the control group, 14 cows (7%) with cystic ovaries had 124 estrous periods. Not including the cystic ovaries, 0.8% of the 368 estrous cycles were less than 18 days. The normal interval of 18 to 25 days included 63.3%, and 35.9% of the cycles had longer intervals than 25 days. The number of silent estrous periods and other ovarian conditions were not known for the cows in the control group.

In Figure 1 are shown the intervals for recurring estrus for cows in the experimental group. The mode for the cycles of the experimental group was 21

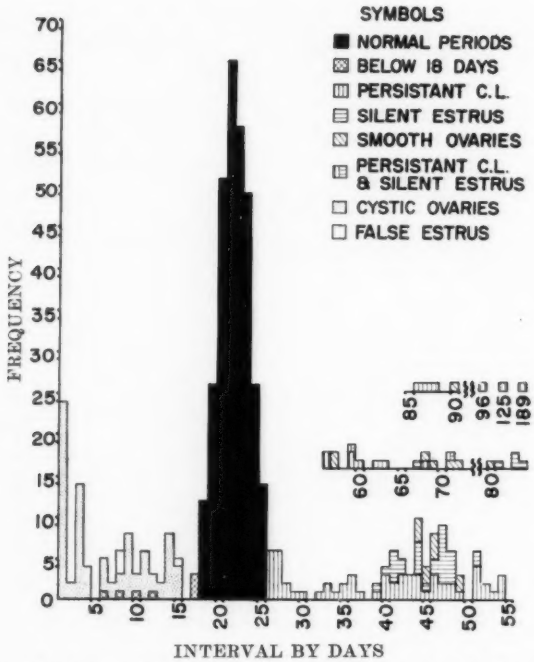

FIG. 1. Histogram showing the intervals of recurring estrus for cows in the experimental group.

days, but the number of cows at intervals of 22 days was nearly as large. For the control group the mode was at 22 days, with the column for 21 days nearly as large, and the number of cycles listed at 20 days was considerably larger than those at 23 days. The mode is much more important than the mean, which was about the same for both groups; the actual from ovarian examinations showed an interval averaging 5.1 days less than the apparent of 29.3 days, determined by external indications of estrus for cows in the experimental group.

There were no differences between the intervals for recurring estrus among the cows receiving service and those with cycles from estrus without service, for either the experimental or the control group. This can be expected in a herd with high fertility and relatively free from any apparent infections in either the males or females. The high level of reproductive efficiency that prevailed for the cows in the control and experimental groups, respectively, was indicated by the following: services per conception to fertile cows 1.73 and 1.70, to all cows 1.90 and 1.91; per cent sterile 3.0 and 4.0; number of abortions and interrupted pregnancies 6 and 12 (3.09 and 6.25%) among 194 and 192 cows which conceived. Chapman and Casida *(5)* report considerable differences between copulatory and noncopulatory cycles. Olds and Seath *(13)* report a difference of 5.1 days for the cycle between cows bred and not bred, but this was in a herd which must be considered borderline on fertility.

TABLE 3
Conception rates in cows bred during and following various ovarian conditions

Estrus and ovarian condition	Cows bred	Conception from one service
	(No.)	*(%)*
Cows bred in control group	200	60.00
Cows bred in experimental group	200[a]	65.00
Artificially bred during silent estrus	20	65.00
Normal estrus after silent estrus	48[b]	62.50
Estrus after persistent corpus luteum	46	65.22
Estrus after smooth, nonfunctional ovaries	15	80.00
Service after recovery from cystic ovaries (expt.)	20	40.00
Service after recovery from cystic ovaries (control)	14	42.86
Service for estrus with cycle less than 18 days (expt. and control) [c]	36	33.33

[a] Some of the cows in this group appear again in one of the other groups.
[b] The total in this group exceeds the number listed for silent estrus after the first postpartum heat in Table 2 because 20 of the 35 cows which had 49 silent periods before the first externally indicated estrus were among the 28 cows which conceived on the first service during the first externally indicated estrus after parturition.
[c] Includes estrous cycles less than 18 days, without cystic ovaries, but usually during estrus before the start of the cystic condition, and therefore not listed in Table 2. Cows bred with these short intervals of estrus often followed with frequent estrus and thus were eventually listed among the cows in the group with cystic ovaries.

Conception rates from service during and following various ovarian conditions are presented in Table 3. Among the 200 cows bred in each of the control and experimental groups, 60 and 65% conceived, respectively. In the experimental group, 20 cows were bred artificially during a silent estrus when they refused to stand for either a cow or bull, and 65% conceived. When service was given during a normal estrus that followed a silent estrus, 62.5% of 48 cows conceived. Forty-six cows were bred during estrus that was delayed because of persistent corpus luteum, and 65.22% conceived.

Service to 15 cows during estrus after delayed estrus due to smooth, nonfunctional ovaries resulted in 80% conceptions. This higher rate of conception is not significant statistically but is in agreement with practical observations and with results from the experiment at Cornell with three levels of nutrition on which heifers in a state of malnutrition often maintained smooth ovaries for long periods of time. When these ovaries eventually became functional, a high rate of conception resulted.

Service after recovery from cystic ovaries, following the best recommended veterinary treatment known today, to 20 cows in the experimental group and 14 of the control cows, resulted in 40 and 42.86% conceptions, respectively. This decreased rate was statistically significant for the experimental group and highly significant when the 34 cows that had recovered from cystic ovaries were considered together. Among the cows in the experimental group that had cystic ovaries 15% were sterile, and the fertile cows required 3.12 services per conception. In the control group, 14.3% of the cows were sterile, and the fertile cows required 3.17 services per conception. These results agree with research by Casida *et al. (4)*, who reported a conception rate of 38% from one service after treatment for cystic ovaries. VanDemark *et al. (17)* also reported low rates of conception after cycles with short intervals.

The results from this experiment show that high rates of conception are obtained after irregular estrus due to various ovarian disturbances such as silent estrus, persistent corpus luteum, and smooth, nonfunctional ovaries. In view of this, service should be provided during irregular estrus attributed to these conditions or involving longer than normal periods. Cows with estrual cycles of less than 18 days had a low rate of conception (33.3%), which was statistically highly significant from the average for the cows in the control and experimental groups. In view of this, it usually is advisable to withhold service to cows with short intervals between estrus and provide veterinary examination and treatment. For estrus after recovery from cystic ovaries, a poor rate of conception and a higher percentage of sterile cows should be expected until additional progress is made on methods of treatment. However, when cows in this category appear normal, they should be bred, and a lower rate of fertility with some delay in conception for some cows should be accepted.

SUMMARY

A total of 400 dairy cows were alternately assigned at random to an experimental and a control group. Regular ovarian examinations for the experimental group provided information on ovarian function, regularity of estrus, and the influence of these on conception rates. The reproductive behavior, as indicated by external signs of estrus, intervals of estrus, and conception rates, was very similar in the two groups.

The average number of days from parturition to the first indicated estrus was 50.9 days for the control group and 49.4 days for the experimental group. The interval for the experimental group as actually determined by ovarian examinations was 43.7 days because 35 cows had 49 silent (unobserved or quiet) estrous periods before the first indicated heat. At 90 days postpartum, 93.0% of the cows in the control group and 93.5% of the experimental cows had indicated postpartum estrus. These figures are based on observing cows for heat in an exercise lot twice daily. Information from ovarian examinations showed that actually 97% of the experimental cows had an estrus at 90 days or less after calving.

When all 500 intervals of estrus were considered for the 200 cows in the experimental group, it was found that 60.4% of the intervals were between 18 and 25 days. A total of 37.4% of the intervals were over 25, and 2.2% were less than 18 days, when estrous cycles indicated from cystic ovaries were excluded. Irregular intervals were due to 18.6% silent estrus, 15.2% persistent corpus luteum, 3.6% smooth, nonfunctional ovaries, 0.8% of false estrus (estrus without the development of a follicle and/or estrus not followed by the development of a corpus luteum), and 1.4% with an interval of less than 18 days.

Low conception rates prevailed after a period of recovery from cystic ovaries and from services for intervals less than 18 days. A high rate of conception (over 60% from one service) was obtained for the cows in the entire group, for cows bred during a silent estrus, during a normal estrus after a silent period, and during delayed estrus due to persistent corpus luteum and smooth, non-

functional ovaries. Breeding cows with irregular long cycles should not be restricted, as is sometimes advocated. The results on breeding efficiency indicated that the chances for conception in cows returning at irregular intervals are not decreased except for cows with short intervals and cows with cystic ovaries. After the cows are treated for cystic ovaries, the conception rate is decreased and the percentage of sterility increased, but given a sufficient number of services the cows will usually conceive.

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EFFECT OF PROGESTERONE THERAPY ON EMBRYO SURVIVAL IN COWS OF LOWERED FERTILITY[1]

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Embryonic death is a direct cause of lowered fertility in repeat breeding cows. Tanabe and Casida *(10)* observed that 66% of the repeat breeding cows with no clinically detectable abnormality had fertilized ova. At 34 days the per cent having normal embryos had decreased to 23. Hawk *et al. (5)* found that 58% of similar repeat breeding cows had embryos 16 days after breeding but only 28% of the cows had normal embryos at 34 days. The cause or causes of embryonic death are not known. A possible explanation could be a deficiency of progesterone, either as a result of decreased luteal function or of the regression of the corpus luteum.

Important questions are whether the embryo dies first and then the corpus luteum regresses or whether regression of the corpus luteum causes the death of the embryo. If the cause of embryonic death is a subminimal level of progesterone, the embryo might die first and then the corpus luteum regress. However, if regression of the corpus luteum causes the death of the embryo, the embryo might die during the regression of the corpus luteum. Theoretically, then, the embryo might be maintained in the absence of the corpus luteum by progesterone therapy beginning before its regression.

Herrick *(6)* and Dawson *(3)* concluded that progesterone therapy in cows which had not settled after repeated breedings was beneficial. In Herrick's work 35% of the treated animals settled on the first service compared to 5% of the controls and in Dawson's 47% of the treated animals settled as compared to 17% of the controls. Both of these control percentages appear low in the light of the observations of Tanabe and Casida *(10)* and Hawk *et al. (5)*.

Certain experimental work pointed out the possibility of a deficiency of progesterone in the repeat breeder cow as a cause of embryonic death. It had been shown by Rowson *et al. (9)* in cows and by Black *et al. (1)* in rabbits that there is very little growth of certain bacteria when infused into the uterus of ovariectomized animals. They also reported that injections of progesterone in the ovariectomized animals promoted conditions conducive to bacterial growth. Thus,

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[2] Agent of the Dairy Husbandry Research Branch, USDA.

it would seem that progesterone in some way inhibits the normal defense mechanism of the animal and allows bacterial growth.

Black *et al. (2)* showed that when semen or bacteria were placed in the uterus of virgin heifers and repeat breeders during the luteal phase, there was a tendency for a differential response. In the virgin heifers there were extensive accumulations of pus from the semen in some cases, whereas in repeat breeders there were only small amounts of pus, or pus was entirely absent. The number of bacteria recovered from the first service animal was, in most cases, larger than the number recovered from the repeat breeder. They postulated that if progesterone inhibits the defense mechanism, then one possible explanation for the difference shown between repeat breeder cows and first service animals would be a deficiency of progesterone in the repeat breeder animal.

Although lowered progesterone function might be the cause of embryonic death, cows could fail to respond to progesterone therapy if the uterus was refractory to progesterone or if the progesterone was metabolized before reaching the uterus. The present study was designed to determine the effects of exogenous progesterone on embryonic survival in repeat breeding cows.

MATERIALS AND METHODS

The animals used in this study were obtained through the cooperation of five artificial breeding organizations; they were purchased directly from the farms and brought to the University by truck. The animals had to meet the following specifications: bred a minimum of four times; bred unsuccessfully within the last 90 days; under 10 years of age; no noticeable purulent discharge; normal to long return intervals following breeding, and no gross abnormalities detectable upon palpation which would hinder fertility. Not more than two cows were purchased from any one farm.

The cows were kept in a pen-type barn. They were fed mixed hay, grass silage, and a grain mixture. Animals that were still giving a small amount of milk when purchased were milked on a once-a-day basis. Heat checks were made twice daily.

The cows as they came into heat were allotted at random as either a control or a treated animal. Cows were bred approximately 12 hours after they were first observed in heat. A few control animals that returned to heat were reassigned and treated.

The injections started 3 days after the beginning of heat and continued until the time of slaughter. Treated animals were slaughtered as soon as the corpus luteum started to regress or, if it did not regress, 34 days after heat. Control cows that did not return to heat were slaughtered 34 days after heat. The control cows returning to heat were rebred and slaughtered. The genital organs in all cows were carefully examined at slaughter for gross abnormalities, and all animals with abnormalities that would obviously hinder fertility were not included in the experimental data.

McDonald *et al. (8)* maintained pregnancies in cows from which the corpus luteum had been removed by daily injections of 100 mg. of progesterone. It

was believed that 50 mg. of progesterone per day supplemental to the animal's own corpus luteum should be sufficient to maintain early pregnancy, but when no great improvement was noted the dosage was increased to 200 mg. per day.

Treatment with 50 mg. of progesterone. Seventy-two cows were used in this part of the study. There were 38 Holstein, 27 Guernsey, three Jersey, two Shorthorn, and two Brown Swiss. The breeds were distributed fairly evenly between the control and treated groups. The cows averaged 5.5 years of age. All animals had calved at least once and averaged 2.8 times. Since last calving, the control animals averaged 7.3 breedings and the treated cows, 6.8. Five animals were positive to the brucellosis test in both the control and treated groups, and 26 control and 19 treated animals were negative to the brucellosis test. The remaining animals in each group were suspects.

The treatment consisted of daily subcutaneous injections of 50 mg. of progesterone dissolved in 2 ml. of corn oil. The genital organs of the cows were palpated sometime during the second week after breeding to locate the corpus luteum. Beginning on day 17 they were palpated every second day until day 25 to determine if and when the corpus luteum regressed.

Treatment with 200 mg. of progesterone. Fifty-four animals were used in this part of the experiment. Eight animals had an infertile control insemination and were rebred and treated, making a total of 62 experimental services (31 treated and 31 control). The animals averaged 7.2 years of age, and the 46 cows had calved an average of 2.4 times. Since last calving, the treated animals had been bred an average of 7.9 times and the control cows, 6.6 times. Three cows were positive to brucellosis in both the treated and the control groups. Seven were suspects among the treated animals and three in the control group; the rest of the cows were negative.

Treatment consisted of daily injections of 200 mg. of progesterone in 8 ml. of corn oil. Each daily dosage was given subcutaneously in at least two sites in the neck. Genital organs of the cows were palpated per rectum sometime during the second week after breeding to locate the corpus luteum. Palpations to determine if and when the corpus luteum regressed were not carried out on a systematic basis, as was done during the 50 mg. study.

RESULTS

Sixteen of the 36 cows (44.4%) that received 50 mg. of progesterone per day had normal embryos at 34 days as compared to 12 (33.3%) in the 36 control cows (Table 1). There were four dead embryos in the control group when the animals were slaughtered 34 days after heat. No dead embryos were found in the treated group; however, one treated cow had degenerate membranes in her uterus at the time of slaughter 34 days after heat. Corpora lutea (apparently functional) were present in all those cows with abnormal embryos or degenerate membranes.

Of the 31 cows that received 200 mg. of progesterone per day, 12 (38.7%) had normal embryos at 34 days, compared to eight (25.8%) of the 31 control

TABLE 1

Effect of progesterone administration to repeat breeder cows upon the number and per cent of cows with embryos at day 34

	No. of cows	No. normal embryos	% normal embryos	No. dead embryos	Degenerate membranes
50 mg. of progesterone					
Treated	36	16	44.4	0	1
Control	36	12	33.3	4	0
200 mg. of profesterone					
Treated	31	12	38.7	0	3
Control	31	8	25.8	1	1
Both treatments					
Treated	67	28	41.8	0	4
Control	67	20	29.8	5	1

For 50 mg. dosage $\chi^2 = 0.52$, $P \cong 0.90$.
For 200 mg. dosage $\chi^2 = 0.66$, $P \cong 0.90$.
Combined $\chi^2 = 1.58$, $P \cong 0.70$.

animals (Table 1). One dead embryo was found in a control cow slaughtered 34 days after breeding. None was found in the treated animals.

Degenerate embryonic membranes were present in the uteri of one control cow and three treated cows. The three treated cows were slaughtered after the corpora lutea had regressed. These cows were slaughtered 32, 33, and 34 days after heat. The membranes in these cows had degenerated to a point where it was difficult to recognize them and they were surrounded by an appreciable accumulation of pus. Small amounts of pus were found in the uteri of three other treated animals that were slaughtered 28, 29, and 34 days after heat. The corpora lutea in these animals had regressed prior to slaughter.

The differences in neither the 50 mg. trial (11.1%) nor the 200 mg. trial (12.9%) were statistically significant. When the data from the two dosage levels were combined, the numbers of normal embryos were 28 (41.8%) and 20 (29.8%) for the treated and control animals, respectively. The difference between these values also was not significant.

No animals that received progesterone injections returned to heat during the course of the injections. No normal embryos were maintained in the treated animals in the absence of a corpus luteum. As soon as the corpus luteum began to regress there was a great deal of follicular development, and the tone of the uterine horns approached that found at the time of estrus. Both of these conditions were more pronounced in the animals treated with 50 mg. of progesterone per day than in those treated with 200 mg. per day.

As far as could be determined, the 50-mg. dosage of progesterone had no significant effect on the time that the corpus luteum began to regress. Records were available on 33 of the treated cows and 34 of the control cows. In 57.6% of the treated animals the corpora lutea had not started to regress by day 25. In 3.0% the corpora lutea started to regress at the 17th day, 33.3% at the 19th day, 3.0% at the 21st day, and 3.0% at the 23rd day. The corpora lutea had not started to regress in 50.0% of the control animals by day 25. In 2.9%

the corpora lutea had started to regress at the 17th day, 29.4% at the 19th day. 11.8% at the 21st day, and 5.9% at the 23rd day.

DISCUSSION

The proportion of treated cows with normal embryos 34 days after breeding was not significantly greater than the proportion of control cows. The magnitudes of the difference were consistent in the two parts of the study and were 11.1% in cows treated with 50 mg. of progesterone per day and 12.9% in cows treated with 200 mg. per day.

The results of the experiment are equivocal. It may be that the difference, although small and nonsignificant in this study, is real. A larger number of cows than is now practical to use at the Experiment Station would be needed to establish significance with such a small difference. Undoubtedly repeat breeding cows as a group are heterogeneous as to the causes of embryonic death. Therefore, an improvement of this magnitude may be all that could be expected from correcting any one cause of repeat breeding.

Opposed to this point of view is the failure of therapy to maintain an embryo beyond the time of corpus luteum regression. This might suggest that something other than the supply of progesterone is the limiting factor in the maintenance of these pregnancies. Finding apparently functional corpora lutea in a few control animals accompanied by dead embryos and degenerate membranes also fails as evidence that the death of the embryo was caused by regression of the corpus luteum. Rather it would seem that the death of the embryo is the cause of regression of the corpus luteum. This is still consistent with the possibility that progesterone therapy may keep some embryos alive (that otherwise would have died because of progesterone deficiency) and they in turn may be responsible for maintenance of the corpus luteum even if only at a low functional level.

SUMMARY

Sixteen repeat breeder cows out of 36 (44.4%) that were injected with 50 mg. of progesterone per day beginning 3 days after heat had normal embryos at 34 days as compared with 12 (33.3%) in an equal number of uninjected control animals. Thirty-one additional animals were injected daily with 200 mg. of progesterone. Twelve of these animals (38.7%) had normal embryos at 34 days as compared with eight (25.8%) in 31 control animals.

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VARIATION IN THE EXCRETION OF CHROMIC OXIDE BY RUMINANTS

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Compared to the conventional method of determining digestibility, the ratio technique offers many advantages and recently has been employed frequently to determine the digestibility of nutrients and to estimate the nutrient intake of grazing animals. Chromic oxide, plant pigments, and lignin have been used for this purpose as the index substance, and among them chromic oxide is used most frequently because of its accuracy and simplicity.

In 1952, Kane *et al. (7)* reported that there was a diurnal variation in the excretion of chromic oxide by dairy cows under normal conditions. The chromic oxide excretion curve indicated that there was only one peak per day in spite of feeding twice daily at 4:30 A.M. and 1:30 P.M., and they assumed that this fluctuation occurred because of some unfamiliar internal biological laws.

Reid *et al. (5, 8)* studied the chromic oxide excretion-time pattern by both grazing and hand-fed steers. In grazing animals the peak concentration of chromic oxide in the feces appeared at night, but in hand-fed animals the maximum value appeared during the day as in Kane's *(7)* experiment. In Reid's *(5, 8)* experiments chromic oxide was administered only once daily, at 7:00 A.M. Reid explained this difference in time of peak excretion of chromic oxide as follows: Clipped grass was given to hand-fed animals twice daily, at 6:00 A.M. and 4:00 P.M. Grazing animals ingested most of their feed from 8:00 P.M. to 6:00 A.M., with only a small amount of grazing outside this period *(2, 4)*. Since ingestion time differed, the excretion curve also differed. The above-mentioned reports indicate that a diurnal variation in the excretion of the chromic oxide is a normal phenomenon in ruminants.

For the maximum accuracy of the ratio technique, it is important to obtain representative samples, and it is desirable to understand the cause of these variations in the chromic oxide excretion.

The experiments reported in this paper were performed to examine the effect of time of feed ingestion and kinds of feed on the diurnal variation of chromic oxide excretion. The daily variation of chromic oxide concentration in the feces was also examined.

EXPERIMENTAL PROCEDURE

Four goats were employed in Trial 1. The goats were kept in the metabolism cages during the experimental period. After the diet containing chromic oxide was administered for about 10 days, each passage of feces for the following 48 hours was collected, weighed, and analyzed for dry matter and chromic oxide.

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Each goat was given 800 g. of hay and 400 g. of concentrate daily. Hay was chiefly composed of orchard grass and some red clover. The concentrate mixture consisted of 5% fish meal, 15% soybean oil meal, 20% ground corn, 30% wheat bran, 27% rice bran, 2% $CaCO_3$, and 1% NaCl. Chromic oxide (0.5%) was thoroughly mixed with the concentrate. The diets were fed twice daily, at 9:00 A.M. and 4:00 P.M., during the experimental period.

Three goats were employed in Trial 2, in which experimental conditions were similar to Trial 1 except that 1,000 g. of concentrate alone was fed. Three goats were employed in Trial 3. The feeding time was at 9:00 A.M., and the feces collecting time was shortened to 24 hours. The feed intake and other experimental conditions were similar to those of Trial 1. Three goats were employed in Trial 4, which was conducted to examine the daily variation in chromic oxide excretion. Hay and concentrate containing no chromic oxide were fed for several days, after which chromic oxide was mixed with the concentrate. The collection of feces was begun when the chromic oxide was first fed. The total feces were collected at 9:00 A.M. each day, mixed thoroughly, and sampled for the analysis of dry matter and chromic oxide.

The chromic oxide was determined by colorimetry at 370 mμ with the spectrophotometer *(3)*.

RESULTS AND DISCUSSION

Each passage of feces was weighed and sampled as soon as it was voided during 48 hours in Trials 1 and 2. However, the number of passages voided ranged from 26 to 44 per 24 hours, and the amount voided at one time varied markedly from 5 g. to 70 g. (on the fresh basis). Moreover, the interval from one passage to another ranged from a few minutes to about 2 hours. When a small amount of feces of high chromic oxide concentration was excreted at rather long intervals, it caused a high peak, which may not be real, when the concentration of chromic oxide in each passage was plotted on a graph. In order to avoid the difficulty, the amount of feces and concentration of chromic oxide voided per each hour period was calculated. Therefore, the time of feces excretion differed a little from the actual time. The data are presented in Figure 1.

As shown in Figure 1, the chromic oxide excretion curves were roughly similar. There was only one high peak each day in spite of twice a day feeding. These results are in agreement with those of Kane *(7)* and Reid *(5, 8)*.

An analysis of variance of the chromic oxide content of feces of animals No. 3 and No. 4 revealed a highly significant difference between the first 24-hour period and the succeeding 24-hour period. On the other hand, there was no significant difference between animal No. 1 and No. 2 in this respect. It is important to determine whether this difference was a normal occurrence, and this point was pursued in Trial 4.

In Trial 2, since the concentrate contained a small amount of indigestible nutrients, the total amount of feces excreted was small. For these reasons, the samples were combined for 2-hour intervals, as shown in Figure 2. Of course,

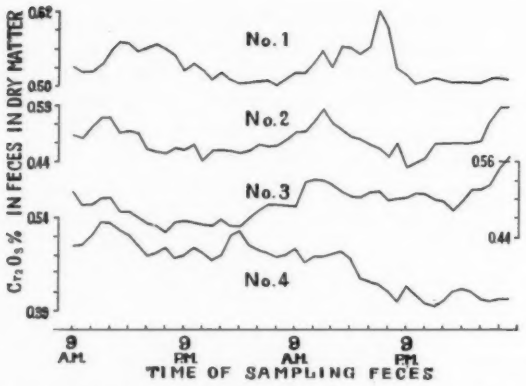

FIG. 1. Chromic oxide excretion curves over a 48 hr. period when both hay and concentrate were administered twice daily at 9:00 A.M. and 4:00 P.M.

these data were obtained from the calculation similar to that used for Figure 1. Despite changing the kind of feed, the chromic oxide excretion curves differed only slightly from those of Trial 1. From the data presented in Figures 1 and 2, it is evident that the movement of chromic oxide through the digestive tract was not affected by the type of ration fed since the curves were very similar.

In Trial 3 the effect of ingestion time was examined by feeding at 9:00 A.M. and 9:00 P.M. As shown in Figure 3, the curve of animal No. 2 differed a little from those of the two other animals. A slight peak appeared at 4:00 P.M., but in general a moderate curve is shown in the earlier part, and at 1:00 A.M. a remarkable peak appeared. In both No. 3 and No. 4 two clear peaks appeared, one in the day and one at night. The results obtained in Trials 1, 2, and 3 demonstrated that the time of feed ingestion influenced the excretion rate of chromic oxide in the feces.

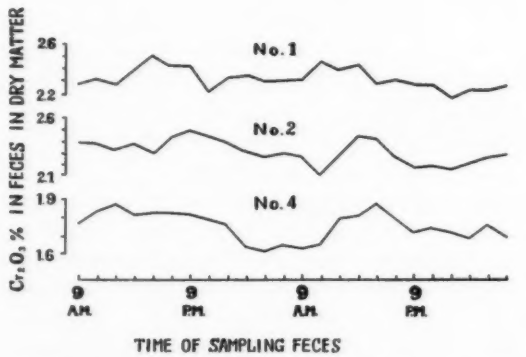

FIG. 2. Chromic oxide excretion curves over a 48 hr. period when concentrate alone was fed twice daily at 9:00 A.M. and 4:00 P.M.

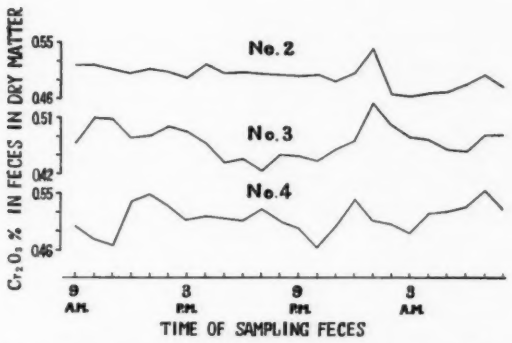

FIG. 3. Chromic oxide excretion curves over a 24 hr. period when both hay and concentrate were fed twice daily at 9:00 A.M. and 9:00 P.M.

On the other hand, in our study of the disappearance of the chromic oxide from the rumen *(6)*, the concentration of chromic oxide in the rumen decreased during the first 3 hours after it was given to about 70% of initial concentration and then maintained this level until the next feeding.

This indicated that chromic oxide was more quickly removed from the rumen than the usual constituents of feed. When chromic oxide contained in the feed was administered twice daily to ruminants, two peaks might be expected to appear in diurnal variation of chromic oxide excretion. However, in Trials 1 and 2 only one peak appeared instead of two. It is presumable that chromic oxide moved through the digestive tract in a manner characteristic of chromic oxide and independent of the feces, and the peak of the chromic oxide excretion curve when it was fed at 4:00 P.M. doubled with the peak when it was fed at 9:00 A.M. When the interval of feeding was always exactly 12 hours, as in Trial 3, it seemed that the chromic oxide excretion curves appeared individually, and consequently two peaks appeared. If this assumption is true, the daily variation in total amount of chromic oxide excretion in Figures 1 and 2 would be expected to be larger than that of Figure 3, but, as shown in Table 1, this was not true. To ascertain the correctness of the above assumptions, further study will be needed.

As shown in Figure 1, the level of chromic oxide excretion during first 24 hours differed from that of the next 24 hours with animals No. 3 and No. 4. Trial 4 was performed to determine whether these differences were due to normal variation. The results are shown in Table 2. When chromic oxide mixed in the feed was given, it took only 3 or 4 days to reach the relatively constant chromic oxide concentration in the feces. This indicated that in goats the amount of chromic oxide fed was almost all excreted after only 3 or 4 days.

In animal No. 2 on the seventh day a relatively high value of chromic oxide appeared, compared with the mean of chromic oxide concentration of feces in the last 10 days, and the low level appeared on the eighth day. In animal No. 3,

TABLE 1

Means of the concentration of chromic oxide in the feces and its standard deviation

	Animal No. 1	Animal No. 2	Animal No. 3	Animal No. 4
Trial 1	*(%)*	*(%)*	*(%)*	*(%)*
First 24 hr.	0.533 ± .024	0.470 ± .019	0.473 ± .025[a]	0.495 ± .018[e]
Second 24 hr.	0.533 ± .029	0.479 ± .025	0.512 ± .019[b]	0.435 ± .031[d]
48 hr.	0.533 ± .092	0.475 ± .021	0.492 ± .029	0.466 ± .034
Trial 2				
First 24 hr.	2.348 ± .058	2.375 ± .067[c]		1.771 ± .085
Second 24 hr.	2.305 ± .083	2.263 ± .097[f]		1.746 ± .070
48 hr.	2.325 ± .061	2.318 ± .110		1.759 ± .075
Trial 3				
24 hr.		0.495 ± .018	0.473 ± .026	0.511 ± .027

[a] There are highly significant differences between *a* and *b*, *c* and *d*, and *e* and *f*.

a high level appeared on the ninth and 11th days, and in animal No. 4 a low level appeared on the fourth day and a high level on the 13th day.

From these results, it seemed that the daily variation of concentration of the chromic oxide excretion was a normal occurrence. The following may be considered to be the causes for the variations: *(a)* Even though the feces were collected at the same time each day, their amount varied every day, the amount of this variation usually reaching 10 to 15%. It is possible that a peak which would normally occur the next day might appear earlier when the amount of feces voided was large, and thus a high daily level of chromic oxide excretion would result. On the contrary when the amount of feces voided was small, a low level would occur. *(b)* It is possible that the chromic oxide might accumulate in some parts of the digestive tract, such as the omasum or abomasum, by unknown laws, and the accumulated chromic oxide might be excreted on a certain

TABLE 2

Daily variation of the concentration of chromic oxide in feces (in dry matter)

Day	Animal No. 2	Animal No. 3	Animal No. 4
	(%)	*(%)*	*(%)*
1	0.082	0.072	0.044
2	0.362	0.157	0.296
3	0.435	0.430	0.416
4	0.490 (100.8)[a]	0.448 (97.4)	0.451 (93.8)
5	0.490 (100.8)	0.446 (96.9)	0.466 (96.9)
6	0.490 (100.8)	0.448 (97.4)	0.488 (101.5)
7	0.537 (110.5)	0.449 (97.6)	0.458 (95.2)
8	0.443 (91.2)	0.440 (95.6)	0.485 (100.8)
9	0.463 (95.3)	0.507 (110.2)	0.481 (100.0)
10	0.481 (99.0)	0.442 (96.0)	0.472 (98.1)
11	0.487 (100.2)	0.502 (109.1)	0.495 (102.9)
12	0.484 (99.6)	0.446 (96.9)	0.501 (104.2)
13	0.495 (101.9)	0.473 (102.8)	0.511 (106.2)
Mean value of last 10 days (from 4th to 13th)	0.486 ±.0239[b]	0.460 ±.0247	0.481 ±.0186

[a] The value parenthesized shows the percentage to the mean value of last 10 days.
[b] Standard deviation of mean.

day. Such a possibility was supported by the results of No. 3 and 4 in Trial 1. In our study on the distribution of chromic oxide in the digestive tract of ruminants *(6)*, the concentration of chromic oxide in the omasum might be higher than that of the abomasum, or the concentration of chromic oxide in the abomasum might be higher than that of the caecum.

As judged from the above results of four trials, the sampling method which was used by Andersen and Kane *(1, 7)*, namely, sampling the feces twice a day for several consecutive days, seemed to be adequate to obtain a representative sample.

SUMMARY

When hay and concentrate containing chromic oxide were administered at 9:00 A.M. and 4:00 P.M. or at 7-hour and 17-hour intervals, only one peak of chromic oxide occurred in the daytime. Two of four animals showed a highly significant difference between the concentration of chromic oxide in the feces of one day and the next.

When the concentrate containing the chromic oxide was fed alone, the chromic oxide excretion curves were roughly similar to those when concentrate and hay were fed.

When the feed was fed at 12-hour intervals, two peaks appeared in the excretion of chromic oxide.

The daily variation of chromic oxide excretion was examined, and it was found that the variation was a normal occurrence, amounting to about 10%.

ACKNOWLEDGMENT

The authors wish to express their sincere appreciation to L. A. Moore and J. W. Thomas of the Agricultural Research Service for their revision of this paper.

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Policy for Pricing Milk and Dairy Products

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The material presented in this article is based on the assumption that free movement of milk supplies among markets provides the only sound and effective method of pricing milk and dairy products. In the opinion of the author, any effort to develop an administrative pricing scheme must reckon with this consideration and certain allied truisms. The more important of these are as follows:

1. The terms, price, supply, and demand, if considered separately, become meaningless insofar as the development of sensible price policy is concerned. Experiences have shown that it is futile to tamper with one without also tampering with the other two of the three component parts of the equation.
2. Strong demand and properly functioning markets are imperative to good farm prices.
3. Government purchase and storage of farm commodities do not substitute for markets. Demand reflects added strength so long as warehouses are being filled, but any supplies acquired must eventually be released.
4. Farm output cannot be successfully controlled by extending control measures to only one factor of production. Such a limited control system may be substantiated in theory, but farmers aren't theorists.
5. High prices alone do not result in high net farm income. Net farm income is obtained by subtracting expenses from the product of price per unit and the number of units sold.
6. Farm output in total responds readily to a strengthening demand but not to a declining demand. The output of individual farm commodities responds to both increases and decreases in demand.
7. So long as the principles of democracy and freedom are adhered to, there is no substitute for the judgment of farmers in directing the use of farm resources.

Various examples might be cited to substantiate the validity of the above statements from the futile experiences in the price rigging arena over the last 25 years.

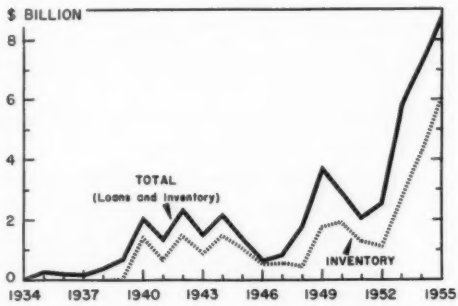

Fig. 1. Value of CCC loans and inventory, 1934-55.

A study of Figure 1 shows how Commodity Credit Corporation holdings have risen to new heights after each event that created circumstances which resulted in temporary inventory reductions. In 1934, which was the first full year's experience with CCC, there were poor crop conditions over much of the country. That year CCC accumulated commodities equal in value to 38 million dollars. In 1935, however, the value jumped to 279 million dollars; but in 1936 with the cooperation of nature, its value dropped to 183 million dollars.

The gleeful moments of the CCC advocates, however, were short-lived. Crop conditions were fairly good during the next few years, and CCC waxed accordingly. In 1940 its value stood at two billion dollars. Although the thinking of farmers had undergone considerable change during the thirties, it had not yet attained a degree of liberalization which could accept this then seemingly vast quantity of surpluses without considerable apprehension. Before any real crisis materialized, however, the smoldering world troubles burst into a flaming conflict. Once again the advocates of the ever normal grainery, which had by now been neatly reenforced with a fine cotton fiber, were a jubilant lot.

On the anticipation of a rapidly rising price level over the next few years, CCC's offering

prices were shifted upward in a couple of hops from the previously employed range of 52-70% of parity (the flexible system, if you please) to 90% by 1943. All was going well and might have been so today except for the declining demand following the close of the war and the fact that someone neglected to change the CCC offering prices accordingly. As is generally true in a competitive society, the purchaser offering the highest price gets the product. CCC was no exception. Its inventory thus attained the then astounding height of 3.7 billion dollars in 1949.

The rapidly accumulating clouds over CCC again started to break away with the outbreak of the Korean conflict. After this conflict was settled, demand began to weaken. Just as before, no one took the occasion to change the buying price scale, and the pitch of the line on the chart from 1952 to 1955 shows the result. CCC now holds under loan and in inventory more than eight billion dollars worth of agricultural surpluses and is still in the buying business.

On the individual commodity basis, the story of stock accumulations is none the less revealing. For example, wheat carry-over increased from 256 million bushels in 1952 to a billion bushels in 1955; cotton carry-over from 2.8 million to 13 billion bales during the same period; and corn carry-over from 487 million bushels in 1952 to more than a billion bushels in 1955. All three are expected to increase again this year.

The dairy industry (with the aid of the Secretary of Agriculture) is to be commended for its foresight (in the relative sense) in acting to adjust support levels before the crisis fully ripened. The wisdom of its action was amply substantiated by ensuing developments.

None of the improvements which followed the change came abruptly, but rather in a gradual manner. Four of the more significant of these changes are as follows:

1. CCC price support purchases of dairy products began in late 1951 (Figure 2).

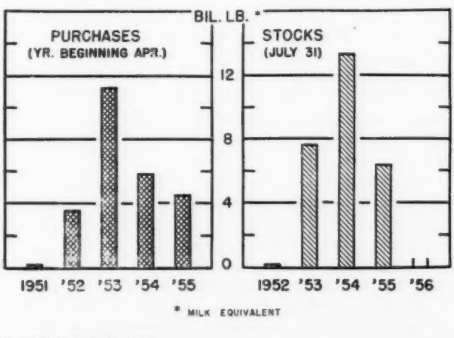

FIG. 2. CCC purchases and stocks of dairy products.

The milk equivalent of purchases for the year 1951 totaled 11 million pounds. In 1952 this figure increased to 3.6 billion pounds and in 1953 jumped to 11.3 billion pounds. CCC purchases for the year 1953 represented 12% of total milk production.

After the reduction in support levels, CCC purchases began to level off and subsequently to decline. Purchases during the year 1954 totaled 5.9 billion pounds (milk equivalent) or about half the 1953 quantity, and in 1955 dropped to 4.4 billion pounds.

Total stocks of dairy products reached a peak of 13.3 billion pounds in 1954 and were reduced to about half this amount in 1955.

2. The consumption of some dairy products—fluid milk and butter in particular—showed slight gains in 1954 and 1955 on the per capita basis. This, coupled with the growth in population, led to a total consumption of some 5% greater than in 1953—prior to the price support change.

3. Milk cow numbers began to increase rapidly, as did total milk production in 1952. After the adjustment in dairy supports, the upward trend in milk cow numbers and production began to subside. These movements and milk production per cow are shown in Figures 3 and 4.

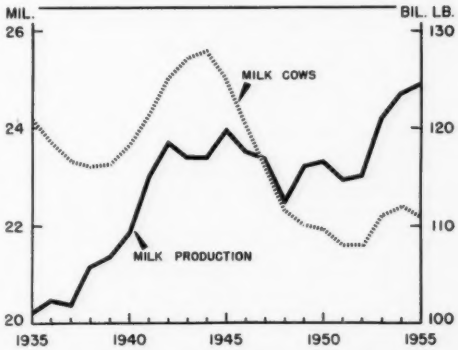

FIG. 3. Milk cows on farms and total milk production, 1935-55.

A part of the change in numbers apparently resulted from closer culling in that the rate of increase in output per cow gained some momentum with the leveling off in cow numbers. Crop and feed conditions were, of course, in the picture too. It is worthy of note in passing that average milk production per cow increased by 1,000 lb. between 1940 and 1955.

Some additional light is shed on the "why's" of these changes in milk production and cow numbers by a study of price relationships between dairy and other farm

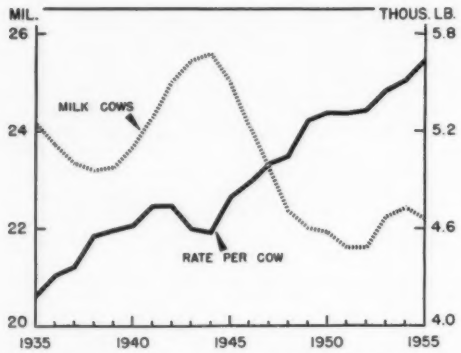

FIG. 4. Milk cows on farms and production per cow, 1935-55.

commodity alternatives. Figure 5 serves to explain this insofar as two of the more closely allied alternatives—beef and pork—are concerned.

From a comparison of this chart with those showing milk production, cow numbers, and surplus accumulation, it is found that there is a high degree of correlation among movements of the four series. During the late 1940's and early 1950's, beef prices were high in relation to milk prices as compared to the relationship of the 1935-39 period. Milk production during this period was fairly stable. Then with the change in price relationships of the two commodities as a result of the decline in beef prices, which made dairying relatively more attractive than in earlier years, came an upward "spurt" in milk production.

Fortunately, higher hog prices developed simultaneously with declining beef prices and effectively served to prevent as full an impact on dairying as might otherwise have been realized.

Changes of the last few months in hog prices are again applying pressure toward an expansion in dairying, as hog prices are now well below the prewar normal relationship with milk prices.

4. Another change indicating greater efficiency in dairying is the current trend toward larger sales per farm and smaller numbers of farms from which milk is sold. These changes are in keeping with the trend toward larger operating units, which has characterized the entire economy of late, but there is reason to believe the rate of change has been gaining momentum since the change in support levels. These changes are shown in Figure 6.

It seems reasonable to conclude that the reduction in the support level of dairy products was a major influence in effecting all the above mentioned changes and that these changes were in the best interest of the dairy industry. Lest some be inclined to doubt this, a comparison may be made between the dairy industry as it exists today and the situation as it existed in April, 1954. Granted all is not as well in dairying today as it might be, the industry is greatly different now from that of 2 years ago when it was characterized by rapidly increasing pro-

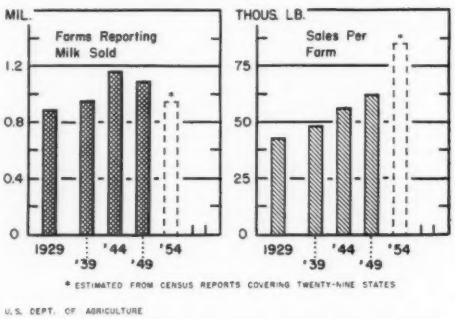

FIG. 6. Number of farms selling whole milk and sales per farm.

duction, declining consumption, and mounting government surpluses.

As we continue to ponder price policy and pricing plans in the future, let us benefit from past experiences. Let us be sure to keep these irrevocable economic principles before us and be directed in our actions accordingly. Let us keep in mind too that dairying is just one of many farm enterprises and that farm resources are to some degree mobile among the various alternatives.

The chances that dairying as a farm enterprise will continue its progress toward a healthier economic situation will be greatly enhanced by taking the following steps:

1. Forego the temptation to write new price legislation as currently advocated by various dairy interests. Experience of the past has clearly demonstrated that about any government price for a farm com-

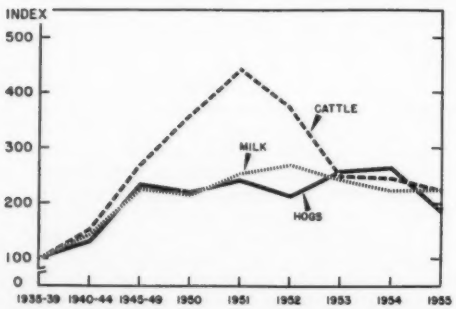

FIG. 5. Farm price relationship—milk, cattle, and hogs, 1935-55. (1935-39 av. = 100)

modity can be legislated but that the will to buy cannot be forced upon the consumer through legislative procedures.

There has been a lot of progress in the development of dairy product substitutes over the last few years. This progress will undoubtedly continue. The industry can, through its price policy, speed up this progress or slow it down.

If there is to be a long life ahead for dairying, the present generation of dairy leaders should not rush headlong into the sacrifice of this future in a futile attempt to attain what might be considered ideal conditions today.

2. Adjust the dairy price support level downward in April, 1956, to 70% of parity as a second step toward placing the dairy industry on firm footing. This will remove some of the luster from dairying as an alternative use for some of the farm resources which will likely be diverted from hogs, poultry, and cattle over the next 2 years as well as to foster continuation of current trends outlined above which are favorable to a more vigorous dairy industry.

3. Direct the concern of all dairy interests toward more aggressive sales programs, the development of higher quality products, greater efficiency in production and marketing, and the assurance of the continuation of a competitive economy.

Competition from the individual's point of view can be a brutal, cold-blooded, merciless thing; but it was through the use of this system that the people of this nation came to enjoy the highest level of living for a longer period of time than any other major country in the history of mankind. However, we should be very cautious in our quest of governmental price making schemes lest in so doing we jeopardize the possibilities of the continuation of free enterprise and thereby "kill the goose that lays the golden eggs."

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BOOK REVIEW

240. Review on the Lecture Outline of Preventive Veterinary Medicine for Animal Science Students. I. A. SCHIPPER.

This book gives a detailed outline of material to be presented in an animal hygiene course for animal science students. The author has divided the material according to systems. The systems discussed are digestive, respiratory, circulatory, urinary, endocrine, and the skin. The physiology and anatomy of each system are briefly outlined, as are the most common related diseases or pathological conditions. Special emphasis is given to epizootiology, systematology, pathogenesis, and pathology of each condition. State and federal regulations and public health aspects are listed when indicated.

In addition, the first chapter contains general information of anatomy, physiology, disinfection, and sterilization and mechanisms of infection and immunity. In the final chapter the most common generalized infections of cattle, sheep and swine, which are not confined to specific systems, are discussed. Special emphasis is given to anthrax, black leg, malignant edema, rabies, swine erysipelas, hog cholera, leptospirosis, and foot-and-mouth disease.

Although the outline is detailed it will not replace the more extensive discussion which a textbook can supply. No discussion of parasitic diseases is given. Parasites are responsible for heavy economic losses in the domestic animals and a discussion of the parasitic diseases therefore should be an important part of a hygiene course. In addition, no reference is made to the common poultry diseases. With the increasing emphasis on poultry some of the most common diseases should be included in a general animal hygiene course.

L. E. Hanson

CHEESE

241. Method of forming Mozzarella cheese. R. A. RUSSO. U. S. Patent 2,733,148. 3 claims. Jan. 31, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 5: 1115. 1956.

A process for making Mozzarella cheese, consisting of cutting the curd under hot water, heating in water to melt the curd, agitating to a smooth consistency after stretching, cutting into blocks, cooling the blocks, treating with brine, partially drying the blocks, and packaging.

R. Whitaker

242. Studies related to the manufacture of filled cheese. I. I. PETERS, Texas Agr. Expt. Sta., College Station. Food Tech., **10**, 3: 138. 1956.

Filled cheese of the cheddar type was made from 200 lb. lots of milk by the conventional method. The milk was separated at 40° C., heated to 55° C., and vegetable fat added in amounts equal to the milk fat removed. The mixture was homogenized at 500 p.s.i., returned to the vat, pasteurized at 61.7° C. for 30 min. and rehomogenized at 500 p.s.i. on the way to the surface cooler. The natural control milk was pasteurized but was not homogenized. Reconstituted skimmilk also was prepared from low heat non-fat dry milk solids (9 lb. to 91 lb. water) and 4% vegetable fat added. This product was processed the same as the filled milk above and made into cheese. Monostearine (0.5 lb/100 lb. fat) or low heat sweet cream buttermilk powder (14 lb/100 lb. fat) was added to the filled milk or to the reconstituted filled milk before pasteurization in certain trials.

The cheese was made in identical 50-gal. cheese vats and the curd from each lot or vat was made into four cheeses of the American favorite style. The paraffined cheese was cured on shelves at 10 to 13° C. and judged at the age of 3 and 6 mo. Bacterial counts, moisture content, fat leakage and pH values were determined on the aged cheese.

Numerical flavor scores of 3- and 6-mo.-old filled cheese ranged from 35 to 37 and for natural cheese from 38 to 40. No significant difference in flavor was noted between filled cheeses made from filled milk or reconstituted filled milk when 3 mo. old, but at 6 mo. the flavor of the filled milk cheese slightly excelled the reconstituted filled milk cheese. The most common flavor criticisms for filled cheese were foreign, flat, and occasionally slightly bitter. Filled cheese containing natural coconut fat showed a lower incidence of bitterness than

filled cheese containing hydrogenated cottonseed oil or soybean oil. The body and texture scores of both the filled cheeses and natural cheese ranged between 26 and 29 but the criticisms were different.

Only the filled cheeses were criticized for being curdy and crumbly but these defects decreased with aging. Ripening in filled cheese tended to progress at a slower rate than in natural cheese as indicated by changes in body and texture and bacterial counts on the cheese after six months' aging. Fat leakage in all filled cheeses was less pronounced than in the natural control cheese made from unhomogenized milk. The pH values of the different lots of filled cheese showed no definite trend. The use of Monostearine in filled cheese showed no advantages at the level tried. The addition of sweet cream buttermilk powder to reconstituted filled milk improved the flavor, body, and texture of filled cheese. E. R. Garrison

DAIRY BACTERIOLOGY

243. Sterilization through radiation. W. M. URBAIN, Swift & Co., Chicago, Ill. Milk Plant Monthly, **45**, 2: 30. 1956.

Beta and gamma rays appear equally effective in their sterilizing actions. Beta rays are secured from generators—high voltage machines built to accelerate electrons. Practical machines so far built have produced electron beams with a penetrability in water of about one inch and since most foods have a density very near that of water, this represents the penetration possible in most foods. Gamma rays are obtained from the radioactive by-products of atomic energy. While a good potential may exist from such sources, today only experimental facilities exist and much needs to be done before commercial utilization of gamma rays will be possible. The sterilizing effect is dependent upon the amount of radiation absorbed, and to secure destruction of all bacteria a definite amount must be provided. The status of this new preservation process is under investigation in many laboratories. It is not used commercially on foods. Its potential value is regarded as great, but irradiation adds a foreign flavor resembling scorching. This coupled with changes induced in the appearance of certain foods has precluded the commercial use of irradiation as a food preservative method. C. J. Babcock

DAIRY CHEMISTRY

244. Determination of specific activity of carbon-14-labeled sugars on paper chromatograms using an automatic scanning device. H. R. ROBERTS and F. J. CARLETON, Natl. Dairy Research Labs., Inc., Oakdale, L. I., N. Y. Analyt. Chem., **28**, 1: 11. 1956.

The quantitative distribution of carbon-14-labeled sugars and sugar derivatives in solutions was determined by chromatographic separation and localization of active sites. The activity of the developed chromatographic strips was determined by an automatic scanning device. This device is applied to the quantitative determination of the specific activity of carbon-14-labeled sugars by two methods. One method employs a summation equation involving the areas under the scanning curves and the total specific activity of the solution being assayed. The second uses known carbon-14-labeled sugar solutions which serve as standards. The heights of the scanning curves of the standards were measured and a straight-line standard curve was prepared. The heights of the scanning curves of the unknowns were measured also, and their specific activities calculated from the standard curve. The application of either of the two methods to the determination of the specific activity of carbon-14-labeled lactose, galactose, and glucose results in an error no greater than 3%. The techniques have been utilized to determine the purity of lactose-carbon-14 derived from a goat. B. H. Webb

245. Two approaches to the detection of substitute fats. W. F. SHIPE, Dept. of Dairy Ind., Cornell Univ., Ithaca, N. Y. Ice Cream Rev., **39**, 7: 64. 1956.

Two methods for the detection of adulteration of dairy products with substitute fats are discussed. One method involves a colorimetric measurement of ferric complexes of hydroxamic acids derived from various fats. The second method involves a selective precipitation of fatty acids with urea.

The author reports that the urea fractionation method is superior to the hydroxamate procedure in the detection of substitute fats in dairy products. The hydroxamate method because of its simplicity has merit as a screening test. It is estimated that the hydroxamate method would permit detection of approximately 20% adulteration. W. J. Caulfield

246. Infrared absorption spectra and protein/sugar inactions. J. D. S. GOULDEN. Nature, **177**, 4498: 85. 1956.

The usual sharp absorption bands in the 1050 cm^{-1} region exhibited by most sugars are replaced by broad bands when various protein/sugar mixtures are examined. The indicated protein/sugar reaction is found in dried milk, in casein/lactose mixtures, etc. It is speculated that a hydrogen bonding mechanism holds the protein and sugar together.
R. Whitaker

247. La solubilisation de la caseine par l'ammoniaque; son utilisation dans la recherche des falsifications du lait frais par addition de lait en poudre. (The solubilization of casein by ammonia; its utilization in study of adulteration of fresh milk by the addition of milk powder.) G. VALLIER. Le Lait, 35: 372. 1955.

To 5 ml. of milk to be tested 4 ml. of commercial ammonia is added, diluted to 50%, and 1 ml. of amyl alcohol then added. The mixture is incubated in a water bath at 77.5° C. for 90 minutes. The resulting color formation in the serum is claimed to be related to the heat treatment of the milk. According to the authors the method is sufficiently sensitive to detect 5% addition of milk powder to fresh milk. It was acknowledged that the previous heat-treatment of the powder was not known.

A. W. Rudnick, Jr.

DAIRY ENGINEERING

248. Automation in the ice cream industry. D. A. SEIBERLING, Dairy Technol. Dept., Ohio State Univ., Columbus. Ice Cream Field, **67**, 1: 20. 1956.

Automation as applied to manufacturing is defined as "making a single machine fully automatic or hooking a number of machines together so they can make something with little human attention."

No instrument is available to make in-the-line determinations of fat and total solids content of dairy products. It is reported, however, that it is feasible to determine, record, and control ice cream overrun, in-the-line, by application of radiation measurements.

It would be difficult to apply automation to a continuous mix-making process unless the types of raw ingredients were restricted. Anhydrous milk fat (99.8% B.F.) and low heat nonfat-milk-solids are suggested as most advantageous for such a process although under proper conditions starting with fluid milk would be a practical source.

Various predictions are made as to possible automation developments. Use of industrial television will permit observation of human activity throughout the plant. Mix processing equipment will be started, stopped, washed, and sanitized under remote control or automatically. Modern accounting and computing equipment can be integrated into this automatic process.

It is emphasized that either major renovation or new construction will be required in converting to automation. The author claims that technical, financial, and public health problems are less serious obstacles to the establishment of automation than the attitude that "it will not work in the dairy industry." He predicts that automation is here to stay.

W. C. Cole

249. Spray drier. S. T. COULTER, W. E. HOYT, and S. VAN DEEST (assignors to Regents of The Univ. of Minn.). U. S. Patent 2,733,762. 13 claims. Feb. 7, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 1: 89. 1956.

A description is given of the important features of the Minnesota spray drier for milk and other dairy products. R. Whitaker

250. Centrifugal liquefier for frozen and solid products. F. L. G. BRYANT (assignor to Bryant Machinery Corp.). U. S. Patent 2,734,502. 12 claims. Feb. 14, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 2: 284. 1956.

A device for rapidly melting frozen cream, butter, etc. consisting of a heated circular drum rotating about its vertical axis. Side walls extend upward to retain the melting products on the drum surface. The melted product flows out to the edge of the drum and is drained off continuously. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

251. Pan-American coffee bureau seminar. Food Field Reporter, **24**, 4: 4. 1956.

Research conducted by the Institute for Motivational Research found that parents often discourage teen-agers from drinking coffee because they resist the growing-up which coffee-drinking symbolizes. Coffee industry has no hesitaney in promoting use of coffee by young people 15 to 19, who now drink some but not enough. Per capita use of coffee by Negroes is less than among whites. Per capita consumption has declined since World War II. An effort will be made to reverse the trend.

F. E. Rice

252. Student participation and use of milk in school lunches. G. G. QUACKENBUSH, Mich. Agr. Expt. Sta., East Lansing. Bull. 252. 1955.

A survey was made of nearly 3,000 schools in Mich. to determine what factors affect participation in school lunch programs. Approximately 35% of the public and private schools participated in the national program in the 1953-54 school year. Nearly 70% of the public school children were enrolled in participating schools. Only 28% of all children took advantage of a lunch program. The total number of lunches served exceeded 6.9 million in Oct., '53, and 99% of these included milk. On a 9-mo. basis, this would equal 31 million lb. of milk or only 0.05% of the state's yearly milk production. Nationally, however, 1% of the milk supply was being consumed in the National School Lunch Program. Factors which appeared to be related to participation rates were: enrollment, type of lunch, price of lunch, availability of chocolate milk, % of children living more than 1 mi. from school, and type of community.

R. W. Hunt

253. Increasing milk consumption in schools. S. W. WILLIAMS et al., Mich. Agr. Expt. Sta., East Lansing. Spec. Bull. 403. 1955.

This survey was made of lunch programs in schools in the 12 N. Central States and Ky. Many children in this region consume insufficient milk for proper nutrition. Milk consumed as a result of the school milk program helps to

overcome this nutritional deficiency. Many of the schools participating in the National School Lunch Program serve a complete lunch in addition to distributing milk. Several schools who do not participate in the NSLP have their own lunch programs. In the year ended June 30, 1954, 75% of the milk served in the Region under the NSLP was consumed as part of a complete lunch. During this same period, 112 million quarts of milk were drunk in Region schools under the lunch program.

Consumption per pupil varied considerably between states and between schools within a state. In schools having an enrollment of less than 1,000, per student consumption varied inversely with enrollment. For schools of more than 1,000 students, rate of consumption was low, but did not continue to decline as enrollment increased. Price of milk, price of lunches, number of students stying for lunch, facilities available for handling milk, and faculty participation in promoting the use of more milk all influenced the amount consumed. However, in most cases, there was no straight-line relationship between these contributing factors and student consumption. The exception to this is faculty interest in promoting the consumption of milk. Milk consumption per student can be increased by the help of teachers.

R. W. Hunt

254. St. Louis, half-gallons and wholesale. T. D. LEATHERS and F. E. SAGESER, Milk Plant Monthly. Milk Plant Monthly, **45**, 2: 14. 1956.

In St. Louis 60% of the milk is sold through grocery stores. Such sales increased 5% in 1955. Half-gallon containers are used for 75% of the milk in the market. They sell for 44¢ each on the retail routes. During the week, the grocery stores sell them for 39¢ each. On Friday and Saturday, it's a common practice to run newspaper coupons and sell half-gallons of milk for 29¢ each—limit one to a customer. Thus a consumer could conceivably pay three prices in a week's time, ranging from 29¢ to 44¢ for the same half-gallon of milk. In a recent 8-month period, paper usage increased 5%, while glass declined the same amount. Paper has 55% of the market. Wholesale represents 60% of the business. At present 5% of the producers are in bulk. They produce 12.3% of the milk. Population in St. Louis increased 7½% in the last four years and milk sales have been increased 11%. Daily per capita consumption has increased from .462 pints in 1940 to .666 pints in 1954. C. J. Babcock

255. Successful bulk conversion. C. L. BURROUGHS and A. D. BURROUGHS. Milk Plant Monthly, **45**, 2: 22. 1956.

The Sunshine Dairy, Paducah, Kentucky, recently went 100% bulk without losing a single producer by: (1) Holding a group meeting with all producers to explain the advantages of bulk pickup, (2) Taking producers on a bus tour to see other producers who had switched to farm tanks, and to hear their experiences, (3) Making a personal visit to each producer, setting up the plan to meet each one's needs, and (4) By setting a special premium price for tank milk, with an agreement for a base rate.

C. J. Babcock

256. Packaging costs are studied for retail selling. C. O. DAVIS, JR., Milk Plant Monthly. Milk Plant Monthly, **45**, 2: 25. 1956.

In a study of packaging costs it was found that the consumer will accept paper containers on retail routes. A survey made among 1,000 families indicated that 52% liked paper, 30% preferred glass, and 18% had no preference. A separate study showed that 90% of the 39 families who quit a dairy said it was because they wanted glass bottles, but 20% of them had not started taking home delivered milk in glass. A later survey of the new customers gained by a dairy showed that the container had very little if any influence. C. J. Babcock

257. Magnesium for new lightweight truck bodies. R. C. SWISHER, Natl. Light Metals and Plastics Corporation, Caro, Mich. Ice Cream Rev., **39**, 7: 38. 1956.

A truck body built of light weight magnesium is described. It is estimated that the use of magnesium will reduce the weight of a truck body by 50% as compared with steel and by 20% as compared with aluminum. In addition to markedly reducing the weight of the truck body, magnesium will produce a more rigid body than either aluminum or steel. A truck body constructed of ⅛-inch thick magnesium sheets offers a high degree of resistance to puncture or impact. W. J. Caulfield

258. Silicones in the dairy industry. C. W. TODD, Dow Corning Corp., Midland, Mich. The Milk Prod. J., **47**, 2: 10. 1956.

Silicones are becoming increasingly important in the dairy industry maintenance and production. Low toxicity, ease of application, heat stability, water repellency, and nonadhesiveness make them ideal aids in many phases of dairy processing including uses as foam inhibitors, release agents, paints, oils, greases, and electric motor insulators. Silicone rubber is gaining increased acceptance as gasketing on autoclaves, oven doors, and steam kettles.

Clear silicone water repellents are excellent maintenance material for brick or masonry structures. Silicones also have applications in the water-repellent treatment of leather, and in veterinary medicines for bloated cattle.

J. J. Janzen

FEEDS AND FEEDING

259. Effects of feeding Systox-treated alfalfa hay to dairy cows. P. A. DAHM and N. L. JACOBSON, Iowa State Coll., Ames. J. Ag. and Food Chem., 4: 150. 1956.

Three experiments were conducted to determine whether demeton or demeton residue toxins fed to cows would appear in the milk or affect the health of the animals. When technical demeton was fed in capsules for 3 consecutive days at the rate of 0.1, 0.5, and 2.5 mg. per kg. of body weight, the cow exhibited severe symptoms of organic phosphate poisoning, milk production dropped sharply, and fat percentage increased. When Systox-treated hay containing an average residue of 51 p.p.m. of demeton toxins was fed to the same cow in increasing amounts for 49 days, mildly adverse effects on weight gains and red blood cell cholinesterase activity were noted. When an average of 41 p.p.m. of Systox-treated hay was fed to three cows for 56 days, no adverse effects on weight changes and milk production were observed, but red blood cell cholinesterase activity decreased gradually during the last 6 weeks of feeding. S. Patton

260. Feedstuffs values and chemical changes in spontaneously heated alfalfa pellets. W. S. RULIFFSON, M. MILNER, and H. L. MITCHELL, Kan. State Coll., Manhattan. J. Ag. and Food Chem., 4: 167. 1956.

Alfalfa pellets from fresh alfalfa hay have become a major feed ingredient. Recently serious losses have occurred, due to spontaneous heating in grain elevators. A comprehensive analytical study, showing progressive deterioration from sound through brown to black pellets, indicated that spontaneous heating is initiated and sustained by sugar-protein interactions of the Maillard type, accompanied by rapid utilization of sugars, slower destruction of fat, and increases in ash, total nitrogen, and crude fiber. In preliminary and intermediate phases, sharp reduction of carotene and regular increases in fluorescence occur. Fat acidity increases rapidly only with onset of advanced browning. Riboflavin values are influenced by fluorescence due to browning products. Retention of nitrogen in heated pellets is attributed to melanoidins resistant to thermal destruction and nutritive utilization. Decomposition is advanced in terms of color, chemical deterioration, and nutritive losses before flaming combustion. S. Patton

261. Effects of feeding different grades of hay and cod-liver oil concentrate to dairy cattle. IV. From birth through one to three lactations. H. B. ELLENBERGER, J. A. NEWLANDER, and C. H. JONES, Vt. Agr. Expt. Sta., Burlington. Bull. 580. 1955.

All calves, heifers, and cows should be fed at a liberal level or enough for thrifty growth, although not necessarily at the maximum rate. It is advisable, and generally economical, to feed only excellent quality roughage. Simple concentrate mixtures are adequate if adjusted to a proper balance with the roughage used. The use of vitamin A and D supplement is not necessary, especially when early cut hay is provided. However, if poor hay is used, a vitamin supplement is advised especially for calves and heifers. R. W. Hunt

262. Carcass characteristics of heavy calves produced by the Kentucky cow-and-calf plan. J. D. KEMP and W. P. GARRIGUS, Ky. Agr. Expt. Sta., Lexington. Bull. 636. 1955.

High quality calves can be raised on milk and pasturage only, with proper feeding and management. Open heifer calves produce beef equal in quality to that of steer calves. Price discrimination against heifer calves is often unjustified. Price discrimination is also found against small calves. These calves, however, have about equal cut-out value and dressing percentage as large calves. R. W. Hunt

GENETICS AND BREEDING

263. Better cows—more profits through improved breeding. L. J. BUSH, E. C. TROUTMAN, and J. R. PERKINS, Ky. Agr. Expt. Sta., Lexington. Cir. 536. 1955.

This bulletin describes how an artificial breeding unit functions, and the benefits derived from artificial breeding. R. W. Hunt

HERD MANAGEMENT

264. Milk cooler. T. B. ROWLAND. U. S. Patent 2,733,048. 3 claims. Jan. 31, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 5: 1093. 1956.

A tank for cooling milk. Refrigeration is provided through a jacket on the bottom of the tank. Agitation is provided through a stream of bubbles rising from a pipe which is inserted into the tank from the top and reaching to the bottom. The rising bubbles impinge on a V-shaped collar which slides on the pipe and is normally located on the surface of the milk. This gives a rolling motion to the milk which facilitates cooling. The tank is closed and under sufficient vacuum to cause the bubbles to rise. R. Whitaker

265. Livestock prod. W. H. ABILDGAARD and C. C. HERBERT (assignors to Livestock Sprayer Mfg. Co.). U. S. Patent 2,733,003. 4 claims. Jan. 31, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 5: 1081. 1956.

A device for prodding livestock, having the appearance of a long barrelled pistol and having a retractable point which provides a mild electric shock to induce the animal to walk. R. Whitaker

266. Cattle squeeze chute. J. P. LA RUE. U. S. Patent 2,733,685. 3 claims. Feb. 7, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 1: 70. 1956.

A device is described for directing, holding, and releasing farm animals. R. Whitaker

ICE CREAM

267. Foil overwrapping transforms ice cream slices into prestige item. ANON. Ice Cream Rev., **39**, 7: 34. 1956.

A new foil-wrapped ice cream package containing either 4 or 16 individual servings has been introduced. Ice cream slices extruded from a continuous freezer pass thru a –45° F. hardening tunnel to a machine which wraps each slice in glassine from a roll. The wrapped slices then are put into chip board cartons by means of a bundling machine. The cartons then go to a machine which applies the aluminum foil overwrap.

The colorful foil overwrap reportedly is ideal from the merchandising standpoint and provides protection against shrinkage and dehydration. It is estimated that the foil overwrap extends the product shelf life 30 to 60 days.

W. J. Caulfield

268. Ice cream making apparatus. J. S. HACKNEY. U. S. Patent 2,731,809. 11 claims. Jan. 24, 1956. Offic. Gaz. U. S. Pat. Office, **702**, 4: 765. 1956.

A vertical-type batch freezer having a refrigerating coil wrapped around the cylinder and a rotating dasher within to provide the necessary agitation to make ice cream.

R. Whitaker

269. Carton for making ice cream sandwiches. R. M. GILL. U. S. Patent 2,734,675. 3 claims. Feb. 14, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 2: 328. 1956.

A carton with a U-shaped liner so constructed that two wafers are held in position to permit soft ice cream from the freezer to be filled into the space between the wafers. A packaged ice cream sandwich is thus produced.

R. Whitaker

MILK AND CREAM

270. Some factors affecting the heat stability and viscosity of half-and-half homogenized milk. E. S. HUMBERT, J. R. BRUNNER, and G. M. TROUT, Dairy Dept., Mich. State Univ., East Lansing. Food Tech., **10**, 3: 134. 1956.

Processing and compositional factors affecting the viscosity of half-and-half milk were investigated. Fresh raw milk was separated at 90° F. and the resultant skimmilk and cream used to prepare six lots of half-and-half homogenized milk. Three lots contained 10.5% fat with additions of 0, 2, and 5% low heat, non-fat dry milk, while the other three lots contained 13, 18, and 23% fat. The samples were pasteurized at 150° F. for 30 min., then portions of each lot were homogenized at 0; 2,500; 5,000; 2,500-500; and 5,000-500 p.s.i. single or double stage in a Manton-Gaulin homogenizer. Half-pint samples of each experimental lot were bottled in glass, cooled in running water to 50° F. and stored at 37° F. Viscosity determinations were made after aging the samples 6 to 10 hr. at 37° F. Heat-stability at 248° F., feathering in hot coffee with calcium ion concentration varying from 0 to 400 p.p.m., and stability at pH levels in the presence of calcium, magnesium, or citrate ion concentrations ranging from 0 to 500 p.p.m. were studied.

Single stage homogenization of half-and-half milk produced much greater increases in viscosity than double stage homogenization, the effect being more pronounced as the fat content increased. The heat-stability of the homogenized samples usually showed an inverse relationship to that of viscosity. The addition of non-fat dry milk generally reduced heat-stability of the product. Increasing concentrations of calcium in distilled-water coffee showed that homogenization of half-and-half milk was conducive to feathering. The acid-stability of the samples was increased by the addition of non-fat dry milk or by the addition of citrate. Magnesium had only a slight effect on acid-stability while the addition of calcium lowered the acid-stability of the samples.

E. R. Garrison

271. Educational drive on milk antibiotics is sought by the Food and Drug Administration. Interview with G. P. Larrick, FDA Commissioner. Food Field Reporter, **24**, 5: 54. 1956.

In the 1955-6 survey, 6.9% of the 1,706 samples of raw and pasteurized milk analyzed showed antibiotic residues, and 5.9% showed only penicillin. It is estimated that 75 tons of antibiotics are used each year for treating mastitis. While FDA requires all packages to be labelled to prohibit use of milk for food purposes for 72 hr. after the cow is treated, it is believed that this caution is widely disregarded. The ave. concentration found was 0.032 units of penicillin per ml., which is not considered harmful except possibly to some individuals who are "exquisitely" sensitive. Nevertheless, FDA is working toward reducing the occurrence. F. E. Rice

272. Cream shipped in bags. Staff Editors, Milk Plant Monthly. Milk Plant Monthly, **45**, 2: 35. 1956.

Thorough field testing has resulted in unqualified approval of handling cream in polyethylene bags at Galva Creamery Co. in Galva, Illinois. Compared to the use of cream cans, the new method saves 25% in weight and 50% in shipping space. The bags are filled with cream at the separator spout and tied shut with a plastic strip. Each bag contains five quarts of cream. They are stored in the farm refrigerator which adds to the product quality.

For shipping, the bags are placed in the insulated corrugated container—four to a container —and are thus protected against heat or cold. Each bag contains 10 pounds of cream so that the 4 bags per carton are about the equivalent of the amount of cream carried in a five-gallon can. C. J. Babcock

273. Technical tips on how to use chocolate products in milk. F. X. KOBE, Rockwood and Co., Brooklyn, N. Y., Food Eng., **28**, 1: 64. 1956.

Both dairymen and soft drink manufacturers have increased their interest in chocolate milk drinks. Factors to be considered in establishing a formula are: Flavor and color of chocolate; acidity and flavor of milk base; solubility and flavor of dried milk powders; sweetness of product; viscosity and sedimentation; fat separation; and sterilization and sanitation. Chief defects of the finished product involve color, sedimentation, and fat separation. Among various precautions, the most practical methods of minimizing these defects are homogenization of the product and the addition of stabilizer. Sterilization of the product by soft drink manufacturers involves heating in the container to 240° F. for 15-20 minutes. This severe process necessitates use of the highest quality ingredients and careful processing to avoid defects. Several formulas for basic syrups and chocolate milk are given. T. J. Claydon

NUTRITIVE VALUE OF DAIRY PRODUCTS

274. Developments in consumer dairy products. H. E. O. HEINEMAN and M. I. WEGNER, Pet Milk Co. J. Am. Dietetic Assoc., **32**, 2: 115. 1956.

In 25 years the per capita consumption of all milk products has increased 9%; however, the increase in use of solids not-fat has been 25%. Improvements in making nonfat dry milk more acceptable for home use, increased interest in frozen desserts of lower fat content, and greater use of milk as whole milk are among the principal factors that have caused this transformation. Among the other new developments are fluid milks with added vitamins and added nonfat solids; sterilized whole milk; 3 to 1 concentrate; low-sodium special prescription milk; dried cream; low fat dairy spreads; and filled ice creams. F. E. Rice

275. Some recent advances in clinical nutrition. J. H. FRYER, School of Nutrition, Cornell Univ., Ithaca, N. Y. J. Am. Dietetic Assoc., **32**, 2: 97. 1956.

Caution needs to be exercised in adding lysine to milk, since it has been found that the addition of amino acids beyond that required in the diet may approach "toxic levels."

Some data support the conclusion that a right combination of cereal and vegetable protein may provide amino acid requirements without the necessity of including animal proteins in the diet. In children with digestive disturbances, showing failure to absorb fat, the fat may not be the offending factor. Many cases of celiac disease have been cured by withdrawing only wheat gluten. Atherosclerosis is caused by a complexity of factors. While some investigators have noted a fall in serum cholesterol when animal fat is replaced by vegetable fat, the withdrawal of animal fats can not be considered important in prevention of the disease. F. E. Rice

276. Beginning the feeding of solid foods in infancy. Acad. of Pediat. Seminar. Pediatries, **17**, 3: 448. 1956.

The nutritional needs of a baby should be considered rather than competition between mothers and doctors to see how soon the baby can begin to tolerate solid foods. There is no need to give the average baby solid foods until it is taking a full qt. of milk a day which on the average is about three months of age. F. E. Rice

277. Bread should have more than lysine. R. BLOCK, New York. Food Field Reporter, **24**, 4: 47. 1956.

Milk proteins contain an abundance of lysine, whereas cereal proteins are deficient. From 100 g. of protein in white, enriched bread the human body is able to make only 20 g. of protein. By adding milk solids and lactalbumen, 100 g. of bread protein will yield 80 g. of body protein. Gelatin is so deficient in tryptophane that it is almost useless used alone in building body protein. With milk, which contains an extra amount of this essential amino acid, gelatin becomes a fairly effective protein. F. E. Rice

278. Acceptability of irradiated foods. Dietary procedures. V. E. MCGARY, et al., Med. Nutrition Lab. Fitzsimmons Army Hosp., Denver. J. Am. Dietetic Assoc., **32**, 2: 123. 1956.

A large number of foods including powdered milk sterilized by gamma ray irradiation were used in meals for 9 subjects (conscientious objectors) over 15-day test periods. Powdered milk was used mostly in cocoa. The irradiated powder had a bitter flavor and a yellow color. However, neither defect was noticeable when the powder was used in cocoa. Later reports will deal with toxicity, metabolizable energy, etc. of the irradiated foods. F. E. Rice

SANITATION AND CLEANSING

279. Today's problems in stream pollution. Ind. and Eng. Chem., **48**, 2: 234. 1956.

Eight papers on phases of stream pollution by chemical plants comprised this symposium.

The behavior of chemical compounds when discharged into a receiving water, their handling in sewage plants, and their effects on the biological life of streams are discussed. Milk and dairy plant disposal problems are not discussed but some of the following papers may be of indirect interest:

"Biochemical Behavior of Synthetic Detergents."
C. N. Sawyer, R. H. Bogan, and J. R. Simpson, Mass. Inst. of Technol., Cambridge, pages 236-240.

"Detergents in Water Supplies."
J. C. Vaughn and R. F. Falkenthal, S. Dist. Filtration Plant, Dept. of Water and Sewers, City of Chicago, Ill., pages 241-245.

"Conditions in Activated Sludge Process During Frothing."
G. P. Edwards and M. E. Ginn, N. Y. Univ., New York, pages 246-250.

"Biochemical Oxidation Characteristics of Stream-Pollutant Organics."
M. B. Ettinger, Robert A. Taft Sanitary Eng. Center, U. S. P. H. Service, Cincinnati, Ohio, pages 256-259.

"Biological Oxidation Parameter Applied to Industrial Wastes."
E. J. Mills, Jr., and V. T. Stack, Jr., Development Dept., Carbide and Carbon Chemicals Co., South Charleston, W. Va., pages 260-262. B. H. Webb

280. Breather pump for cleansing and sterilizing milking lines. H. H. Hill. U. S. Patent 2,733,667. 2 claims. Feb. 7, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 1: 65. 1956.

A device for forcing a cleaning and sterilizing solution through a milking machine system, consisting of a tank holding the solution and a system of pipes and valves which force the solution from the tank by means of a pulsating vacuum supply. R. Whitaker

DIFCO

Sensitivity Testing

of

TUBERCLE BACILLI

BACTO-SENSITIVITY DISKS

Sensitivities to the commonly employed therapeutic agents are accurately and readily determined using Bacto-Sensitivity Disks containing three concentrations of Dihydrostreptomycin, Streptomycin, Viomycin, PAS (p-Amino Salicylic Acid) and INH (Isonicotinic Acid Hydrazide). Concentrated specimen or pure cultures are evenly streaked on Bacto-Peizer TB Medium and Sensitivity Disks applied. Zones of inhibition of growth following incubation indicate if an organism is sensitive, moderately sensitive or resistant to the various chemotherapeutic agents.

Peizer TB Medium recommended for sensitivity test procedures is easily and readily prepared from Bacto-Peizer TB Medium Base and Bacto-Peizer TB Medium Enrichment (Peizer and Schecter: Am. J. Clin. Path., 20:682:1950). This is a selective medium suitable for primary culture and simultaneous sensitivity determinations of tubercle bacilli from concentrated specimen.

BACTO-SENSITIVITY TEST MEDIA

Bacto-ATS Medium and Bacto-Lowenstein Medium are available in screw capped tubes with added chemotherapeutic agents. The ATS Medium is prepared according to the formula specified by the American Trudeau Society; the Lowenstein Medium according to the Jensen modification. These media are processed under carefully controlled conditions to give rapid optimal growth of the tubercle bacilli.

Bacto-ATS Sensitivity Media and Bacto-Lowenstein Sensitivity Media are supplied in sets of 12 tubes consisting of 2 tubes of control medium without therapeutic agent and one tube each of the following:

Streptomycin 10 mcg./ml.	INH 5 mcg./ml.
Streptomycin 100 mcg./ml.	Streptomycin 10 mcg. and PAS 100 mcg./ml.
Viomycin 10 mcg./ml.	Streptomycin 100 mcg. and PAS 100 mcg./ml.
Viomycin 100 mcg./ml.	Streptomycin 10 mcg. and INH 1 mcg./ml.
INH 1 mcg./ml.	Streptomycin 100 mcg. and INH 5 mcg./ml.

The above media are also supplied in packages of 12 tubes of any one medium. In addition, for more detailed determinations, ATS Sensitivity Media and Lowenstein Sensitivity Media containing Streptomycin 250 mcg., Viomycin 250 mcg., and INH 0.2 mcg. are available in packages of 12 tubes.

These media are supplied in incubator packages which permit preferred horizontal incubation.

Bulletin No. 141 describing Sensitivity Testing of Tubercle Bacilli is available upon request.

DIFCO LABORATORIES

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